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# The incidence and outcome of severe sepsis in Finland

## The Finnsepsis Study

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Academic Dissertation

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To Henri and other lights of my life

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This thesis is based on the following original publications, which are referred to by their Roman numerals in the text. The publications are reprinted with the kind permission of the copyright holders.

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II Karlsson S, Pettilä V, Tenhunen J, Laru-Sompa R, Hynninen M, Ruokonen E for the Finnsepsis Study Group. HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis. *Intensive Care Medicine* 2008; 34: 1046- 1053.

III Karlsson S, Pettilä V, Tenhunen J, Lund V, Hovilehto S, Ruokonen E for the Finnsepsis Study Group. Vascular endothelial growth factor in severe sepsis and septic shock. *Anesthesia and Analgesia* 2008; 106: 1820-1826.

IV Karlsson S, Ruokonen E, Varpula T, Ala-Kokko TI, Pettilä V for the Finnsepsis Study Group. Long-term outcome and quality-adjusted life-years after severe sepsis. *Accepted for publication in Critical Care Medicine*

# ABBREVIATIONS

| | |
|---|---|
| ACCP | American College of Chest Physicians |
| ACTH | Adrenocorticotropic hormone |
| AKI | Acute kidney injury |
| ALI | Acute lung injury |
| APACHE II | Acute Physiology and Chronic Health Evaluation |
| ARDS | Acute respiratory distress syndrome |
| AT III | Antithrombin III |
| AUC | Area under the curve |
| AVP | Arginine vasopressin |
| BSI | Bloodstream infection |
| CAP | Community-acquired pneumonia |
| CI | Cardiac index |
| CIRCI | Critical illness–related corticosteroid insufficiency |
| CO | Cardiac output |
| CVP | Central venous pressure |
| DAMP | Damage-associated molecular pattern |
| DIC | Disseminated intravascular coagulation |
| DO2 | Oxygen delivery |
| EGDT | Early goal-directed therapy |
| ELISA | Enzyme-linked immunosorbent assay |
| EQ-5D | EuroQol-5 dimension |
| HES | Hydroxyethylstarch |
| HMGB1 | High mobility group box-1 protein |
| HR-QOL | Health-related quality of life |
| HVHF | High volume hemofiltration |
| ICU | Intensive care unit |
| IL | Interleukin |
| ISTH | International Society for Thrombosis and Haemostasis |
| LOS | Length of stay |
| LPS | Lipopolysaccharide |
| MAP | Mean arterial pressure |
| MODS | Multiple organ dysfunction syndrome |

| | |
|---|---|
| MOF | Multiple organ failure |
| MOS SF-36 | Medical Outcome Study 36-Item Short Form Health Survey |
| MRSA | Methicillin resistant Staphylococcus aureus |
| NE | Norepinephrine |
| NF-κB | Nuclear factor κB |
| NHP | Nottingham Health Profile |
| NO | Nitric oxide |
| PAF | Platelet activating factor |
| PAMP | Pathogen associated molecular pattern |
| PCWP | Pulmonary capillary wedge pressure |
| PRR | Pattern-recognition receptor |
| QALY | Quality-adjusted life year |
| QOL | Quality of life |
| RAND-36 | RAND 36-Item Health Survey |
| rhAPC | Recombinant human activated protein C |
| ROC | Receiver operating characteristic curve |
| SAPS II | Simplified Acute Physiology Score II |
| SARS | Severe acute respiratory syndrome |
| SCCM | Society of Critical Care Medicine |
| ScvO2 | Central venous oxygen saturation |
| sFLT1 | Soluble fms-like tyrosine kinase 1 |
| SIRS | Systemic inflammatory response syndrome |
| SOFA | Sequential Organ Failure Assessment |
| SOFAmax | Maximum Sequential Organ Failure Assessment score |
| SSC | Surviving Sepsis Campaign |
| SVV | Stroke volume variation |
| SvO2 | Mixed venous oxygen saturation |
| SPSS | Statistical Package for the Social Sciences |
| TFPI | Tissue factor pathway inhibitor |
| TLR | Toll-like receptor |
| TNF-α | Tumor necrosis factor-α |
| VAS | Visual analogue scale |
| VEGF | Vascular endothelial growth factor |
| WB | Western immunoblotting |

# ABSTRACT

**BACKROUND** Severe sepsis is associated with common occurrence, high costs of care and significant mortality. The incidence of severe sepsis has been reported to vary between 0.5/1000 and 3/1000 in different studies. The worldwide Severe Sepsis Campaign, guidelines and treatment protocols aim at decreasing severe sepsis associated high morbidity and mortality. Various mediators of inflammation, such as high mobility group box-1 protein (HMGB1) and vascular endothelial growth factor (VEGF), have been tested for severity of illness and outcome in severe sepsis. Long-term survival with quality of life (QOL) assessment is important outcome after severe sepsis. The objective of this study was to evaluate the incidence, severity of organ dysfunction and outcome of severe sepsis in intensive care treated patients in Finland. HMGB1 and VEGF were studied in predicting severity of illness, development and type of organ dysfunction and hospital mortality. The long-term outcome and quality of life were assessed and quality-adjusted life years and cost per one QALY were estimated.

**PATIENTS** A total of 470 patients with severe sepsis were included in the study. Patients were treated in 24 Finnish intensive care units in a 4-month period from 1 November 2004 to 28 February 2005.

**MAIN RESULTS** The incidence of severe sepsis was 0.38 /1,000 in the adult population (95% confidence interval 0.34-0.41). Septic shock (77%), severe oxygenation impairment (71.4%) and acute renal failure (23.2%) were the most common organ failures. The ICU, hospital, one-year and two-year mortalities were 15.5%, 28.3%, 40.9% and 44.9% respectively. HMGB1 and VEGF were elevated in patients with severe sepsis. VEGF concentrations were lower in non-survivors than in survivors, but HMGB1 levels did not differ between patients. Neither HMGB1 nor VEGF were predictive of hospital mortality. The QOL was measured median 17 months after severe sepsis and QOL was lower than in reference population. The mean QALY was 15.2 years for a surviving patient and the cost for one QALY was 2,139 €.

**CONCLUSIONS** This study showed that the incidence of severe sepsis is lower in Finland than in other countries. The short-term outcome is comparable with that in other countries, but long-term outcome is poor. HMGB1 and VEGF are not useful in predicting mortality in severe sepsis. The mean QALY for a surviving patient is 15.2 and as the cost for one QALY is reasonably low, the intensive care is cost-effective in patients with severe sepsis.

# 1 INTRODUCTION

Severe sepsis has long been a challenge in intensive care because of its common occurrence and the high costs of care. Severe sepsis is associated with significant mortality, which has varied between 27% and 55% in different studies (Flaatten 2004; Engel et al. 2007). The incidence or outcome of severe sepsis has not been prospectively studied in the Nordic countries. The incidence of severe sepsis increases annually 1.5% mainly due to the ageing population (Angus et al. 2001 a). Some studies have shown that the prognosis of severe sepsis can be improved (Rivers et al. 2001; Bernard et al. 2001). The international Surviving Sepsis Campaign (SSC) first published guidelines for severe sepsis in 2004 (Dellinger et al. 2004) and an update in 2008 (Dellinger et al. 2008). The main objective of SSC is to decrease mortality by 25% in five years.

The SSC guidelines for severe sepsis treatment are based on studies showing that with relatively simple therapeutic interventions it is possible to save a significant number of lives. Treatments such as ventilation with low tidal volumes (The Acute Respiratory Distress Syndrome Network 2000), low-dose hydrocortisone for vasopressor-resistant septic shock (Annane et al. 2002) and recombinant human activated protein C (rhAPC) (Bernard et al. 2001) have been selected as recommended treatments in the Campaign's Sepsis Management Bundle. Administration of antimicrobial treatment within 3 hours after diagnosis of severe sepsis is recommended (Dellinger et al. 2008). In fact, hospital survival increases if adequate antimicrobial treatment is started within one hour after documented hypotension in septic shock (Kumar et al. 2006). However, recent studies concerning lung protective ventilation have shown that the compliance with the new guidelines into practice is also inadequate in a critical care setting (Young et al. 2004; Kalhan et al. 2006).

Numerous studies have emphasized the important role of proinflammatory cytokines in sepsis. Despite their obvious role in the pathogenesis of severe sepsis, attempts to improve the prognosis of sepsis by inhibiting ‘early’ cytokine-mediated inflammatory process have failed (Opal et al. 1997; Abraham et al. 1998). Since then, novel mediators of severe sepsis as high mobility group box-1 protein (HMGB1) have emerged (Wang et al. 1999). It is speculated that it might be a potential target for anti-inflammatory treatments (Yang et al. 2004). Vascular permeability increases in response to systemic inflammation mediated by

endotoxin and various cytokines. Macrophages and lymphocytes produce vascular endothelial growth factor (VEGF), which is a potent endogenous mediator of vascular permeability (Senger et al. 1983). In addition, VEGF is a potent hypoxia-induced mediator of angiogenesis (Shweiki et al. 1992).

Short-term mortality such as 28-day or 2-month mortality has been used to measure outcome in sepsis studies (Bernard et al. 2001; Rivers et al. 2001; Sprung et al. 2008). Severe sepsis often causes several organ dysfunctions, from which surviving patients may recover slowly. Long-term outcome has been determined in only few studies and one-year mortality has been 51.4% and five-year mortality as high as 74.2% after severe sepsis (Weycker et al. 2003). Nowadays, short-term survival is not the only adequate outcome and long-term survival and quality of life (QOL) have also become an important outcome (Marshall et al. 2005). Poor quality of life before intensive care admission may even predict poor outcome (Hofhuis et al. 2007).

A quality-adjusted life year (QALY) comprises length of life and quality of life. The concept of QALY enables comparisons of the efficacies of different treatments and calculations of costs per QALY. Cost-effectiveness analysis measures benefits of treatments in terms of life years saved. Cost-utility analysis measures treatments using number of QALYs as a unit of efficacy (Anonymous 2002). In intensive care medicine, the generally accepted cost for one life year gained or for one QALY has been $50,000 (Talmor et al. 2006).

In this nationwide study the aim was to investigate the incidence of severe sepsis, associated organ dysfunction and outcome. HMGB-1 and VEGF as mediators of inflammation were studied in the prediction of severity of organ dysfunction and hospital mortality. Special attention was paid to long-term survival and quality of life before and after severe sepsis. As severe sepsis is often associated with high cost of treatment, the quality-adjusted life years were estimated for survivors and cost of treatment was calculated.

# 2 REVIEW OF THE LITERATURE

## 2.1 DEFINITION OF SEVERE SEPSIS

Sepsis is defined as a host's systemic response to infection (Ayres 1985). Different terms such as septicaemia, sepsis or sepsis syndrome were used without precise definitions or even in inflammatory states without infection (Bihari 1990; Marshall et al. 1990). The most widely used definitions of sepsis, severe sepsis and septic shock were published in 1992 by the Consensus Conference of the American College of Chest Physicians (ACCP) and Society of Critical Care Medicine (SCCM) (Bone et al. 1992). The systemic inflammatory response syndrome (SIRS) was introduced to represent the body's systemic response to infection. SIRS criteria consist of changes in temperature, heart rate, respiratory rate or leucocyte count (Table 1).

**Table 1.** The SIRS criteria (Bone et al. 1992).

| **≥ 2 of the following conditions** |
|---|
| • Temperature > 38 ºC or < 36 ºC |
| • Heart rate > 90 beats per minute |
| • Respiratory rate > 20 breaths per minute or pCO2 < 32 mmHg (4.3 kPa) |
| • White blood cell count > 12 x $10^9$/l or < 4 x $10^9$/l or > 10% immature (band) forms |

A microbiologically proven or suspected infection together with SIRS is defined as sepsis. Severe sepsis is sepsis with acute organ dysfunction, hypoperfusion or sepsis-induced hypotension (systolic blood pressure < 90 mmHg or a reduction ≥ 40 mmHg from baseline). Septic shock is defined as severe sepsis with hypotension that persists despite adequate fluid resuscitation or with signs of hypoperfusion (e.g. lactic acidosis or oliguria) in patients with vasoactive treatment. These broad definitions were suitable for clinicians at the bedside to diagnose severe sepsis, but more precise definitions were required, especially for inclusion in clinical trials (Levy et al. 2003). The relationship between infection, SIRS and severe sepsis is presented in Figure 1.

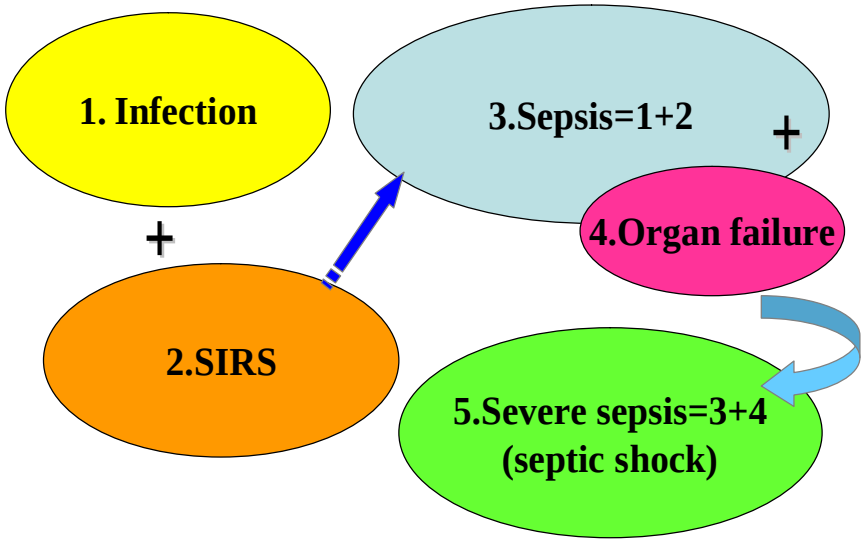



**Figure 1**. Relation of infection, SIRS, sepsis and severe sepsis.

### 2.1.1 Systemic inflammatory response syndrome

Systemic inflammatory response can result from trauma, burns, postoperative states, pancreatitis or autoimmune diseases without proven infection. SIRS criteria were criticized as being too insensitive and unspecific and having no prognostic value. Rangel-Frausto in 1995 published the first study on the epidemiology of SIRS and its continuum to sepsis, severe sepsis and septic shock (Rangel-Frausto et al. 1995). Over 3,500 patients were screened for SIRS in three ICUs and general wards and 68% of the patients met ≥2 SIRS criteria. SIRS was related to confirmed infection only in 50% of patients as SIRS-positive patients were found to have sepsis in 26%, severe sepsis in 18% and septic shock in 4% of cases. In that study, 28-day mortality varied from 7% in patients with SIRS to 46% in patients with septic shock. Patients with infection at admission or during the ICU stay were studied in the European Sepsis Study five years later (Alberti et al. 2002). The mortality varied from 20% to 60% in different sepsis categories, but did not differ between patients with infection and with or without SIRS. Mortality was unaffected by the number of SIRS criteria fulfilled (Alberti et al. 2003). Despite the nonspecificity of SIRS criteria, they have

maintained their position as a marker of systemic inflammatory response (Levy et al. 2003).

### 2.1.2 Organ dysfunction

Existing organ dysfunction or failure is essential for the diagnosis of severe sepsis, but they are not precisely defined in the Consensus criteria (Bone et al. 1992). Sepsis trials have used organ dysfunction definitions of their own (Warren et al. 2001; Brunkhorst et al. 2008) and organ failure criteria used in the Prowess trial (Bernard et al. 2001) have been adapted for other studies as well (Finfer et al. 2004 a; Abraham et al. 2005). The most widely used score for the assessment of organ dysfunction was developed by the working group on sepsis-related problems of the European Society of Intensive Care Medicine 1994 and published 2 years later as the Sepsis-related Organ Failure Assessment (SOFA) score (Vincent et al. 1996). Soon this "sepsis-relation" was renamed "sequential", because these types of organ dysfunctions are not only related to infection. Organ function is graded from 0-4 points (from 0=no dysfunction to 4=severe dysfunction) in six different organ systems (Table 2).

**Table 2.** The SOFA score.

| Organ dysfunction | 0 point | 1 point | 2 points | 3 points | 4 points |
|---|---|---|---|---|---|
| **Respiration** $P_aO_2/F_iO_2$ | > 400 mmHg (> 53.3 kPa) | < 400 mmHg (< 53.3 kPa) | < 300 mmHg (< 40 kPa) | < 200 mmHg (< 26.7 kPa) with respiratory support | < 100 mmHg (< 13.3 kPa) with respiratory support |
| **Cardiovascular** MAP or vasoactive treatment | MAP $\geq$ 70 mmHg | MAP < 70 mmHg | Dopamine $\leq$ 5 µg/kg/min or dobutamine any dose | Dopamine > 5 µg/kg/min or norepinephrine or epinephrine $\leq$ 0.1 µg/kg/min | Dopamine > 15 µg/kg/min or norepinephrine or epinephrine > 0.1 µg/kg/min |
| **Renal** Creatinine or diuresis/24h | < 110 µmol/l | 110-170 µmol/l | 171-299 µmol/l | 300-440 µmol/l or diuresis < 500ml/24 h | >440 µmol/l or diuresis < 200ml/24 h |
| **Coagulation** Tromb | $\geq$ 150 x $10^9$/l | < 150 x $10^9$/l | < 100 x $10^9$/l | < 50 10x $10^9$/l | < 20 x $10^9$/l |
| **Liver** Bilirubin | < 20 µmol/l | 20-32 µmol/l | 33-101 µmol/l | 102-204 µmol/l | > 204 µmol/l |
| **Central nervous System** (GCS) | 15 | 13-14 | 10-12 | 6-9 | < 6 |

Increasing or constantly high SOFA scores are correlated with morbidity and mortality in patients with severe sepsis (Levy et al. 2005), ICU patients in general (Vincent et al. 1998; Moreno et al. 1999), as well as in patients with trauma (Antonelli et al. 1999). There are other organ dysfunction scores, e.g. the multiple organ dysfunction score (MODS), which was introduced one year before SOFA (Marshall et al. 1995). MODS has been shown to correlate with mortality (Jacobs et al. 1999). MODS includes the same six organ systems as SOFA but cardiovascular dysfunction is more complicated to score. Unfortunately, both scoring systems lack gastrointestinal dysfunction, because no suitable descriptor for that organ system was agreed (Marshall et al. 1995). The MODS score is not widely used.

Sepsis definitions were re-evaluated in 2001 but were left practically unchanged (Levy et al. 2003). Additional diagnostic criteria for sepsis were proposed, but none of them was specific for sepsis. However, a new concept for staging sepsis was introduced based on a TNM-classification system in oncology (Denoix 1946). This new system was first called IRO, where I stands for insult (infection), R for response (SIRS, other signs of sepsis) and O for organ dysfunction (SOFA, MODS) (Marshall et al. 2003). After P was added for predisposition (premorbid illness), the concept is called PIRO (Levy et al. 2003). This new PIRO system was left at the conceptual level until the first large study to use the PIRO concept for outcome prediction was published 4 years after this concept was introduced (Moreno et al. 2008). Modified PIRO (organ failure included in response) was tested in SAPS 3 study in patients with infection or sepsis. The SAPS 3 PIRO score was introduced with the ability to predict hospital mortality accurately in patients with infection, sepsis or severe sepsis (Metnitz et al. 2005).

### 2.1.3 Infection

The ACCP/SCCM consensus presented a broad definition for infection as an invasion of normally sterile tissues by microorganisms or as an inflammatory response they cause (Bone et al. 1992). Bacteraemia (correspondingly viraemia, fungaemia or parasitaemia) is defined as a type of infection with viable micro organisms in the blood. Bloodstream infection (BSI) with a positive blood culture is not needed for a diagnosis of sepsis. When over 1,900 ICU patients were evaluated, 92% of them had SIRS, 6% had sepsis with BSI and 3% septic shock with BSI (Laupland et al. 2004). In fact, positive blood cultures are detected in only 20%-55% in patients with severe sepsis or septic shock (Brun-Buisson et

al. 1995; Alberti et al. 2002; Vallés et al. 2003; Ylipalosaari et al. 2006). Abdominal infections are more likely to be associated with positive blood cultures than other foci (Alberti et al. 2002). Infections causing severe sepsis can be microbiologically documented in only 32.5%-71% of cases (Brun-Buisson et al. 1995; Vincent et al. 2006; Moreno et al. 2008, Engel et al. 2007). Hospital-acquired infections are more often microbiologically confirmed (71% vs. 55%) than community-acquired infections (Alberti et al. 2002).

The broad infection definitions were used in many interventional clinical trials that were unsuccessful in demonstrating benefit from experimental drugs in severe sepsis like anti-tumour necrosis factor (anti-TNF) monoclonal antibody trial (Fisher et al. 1993), anti-platelet activating factor (PAF) receptor antagonist trial (Dhainaut et al. 1998) or high dose antithrombin (AT) III trial (Warren et al. 2001). The rhAPC trial with positive results (the Prowess Study) also used modified Consensus criteria (Bernard et al. 2001). However, the heterogeneity of treatment groups in previous trials was suspected to be one reason for the unexpected results. Precise definitions of infection in ICU treated patients with severe sepsis were needed to improve the quality and comparability of clinical trials.

A consensus conference was held in 2003 and the definitions of infection in the intensive care unit were published in 2005 (Calandra et al. 2005). The six most common infection types were identified and described with precise definitions such as pneumonia, bloodstream infections (including infective endocarditis), intravascular catheter-related sepsis, intra-abdominal infections, urosepsis, and skin and soft tissue infections (surgical wound infections or nonsurgical site infections). In addition, each type of infection was classified as being microbiologically confirmed, probable or possible. Large clinical trials or epidemiological studies in severe sepsis published in recent years have not yet been able to use these detailed definitions of infection because patient recruitments have started before 2005. (Abraham et al. 2005; Vincent et al. 2006; Sprung et al. 2008; Brunkhorst et al. 2008). Most recent epidemiological studies have used the old or modified Consensus criteria for infection (Cheng et al. 2007; Engel et al. 2007).

## 2.2 INCIDENCE OF SEVERE SEPSIS

### 2.2.1 Incidence

The first study to show the high incidence of severe sepsis on population level was published at the beginning of this millennium (Angus et al. 2001 a). This large retrospective study screened the data of over 6.6 million hospital discharge records in USA and over 192,000 patients with severe sepsis were identified. The estimated incidence of severe sepsis was 3/1,000 population in 1995, which meant 751,000 patients annually in USA. Half of those patients were treated in intensive care. Another large retrospective study found similar incidence of 2.4/1,000 ten years later (Martin et al. 2003). The incidence was lower in prospective studies with limited study population as in patients treated in ICU. These estimations have varied between 0.51/1,000 and 0.77/1,000 in adult populations (Padkin et al. 2003; Finfer et al. 2004 a). The proportion of patients with severe sepsis in ICU admissions has varied from 9% to 27% (Brun-Buisson et al. 1995. Padkin et al. 2003), but has been around 10%-12% in most studies (Sands et al. 1997; Finfer et al. 2004 a; van Gestel et al. 2004; Engel et al. 2007). In the European SOAP study a notable difference was found on ICU admissions between different countries. Switzerland had the lowest (10%) and Portugal the highest (64%) severe sepsis rates on ICU admission (Vincent et al. 2006). Assuming that only half of the patients with severe sepsis are treated in ICUs, prospective studies still have clearly lower incidence numbers than retrospective studies. However, since most prospective incidence studies have been conducted in ICU-treated patients, the incidence numbers may reflect more ICU beds available for treating severe sepsis than true incidence in population (Linde-Zwirble et al. 2004). The data on sepsis or severe sepsis incidence studies are presented in Table 3.

**Table 3.** Incidence of severe sepsis in different studies. Adm=admission, NA=not applicable.

| Reference | Country | Time frame and design | Severe sepsis definition | No. of patients screened | Screened population | No. of patients | Incidence | Age (years) Male |
|---|---|---|---|---|---|---|---|---|
| Salvo et al. 1995 | Italy | 01.04.1993-31.03.1994 Prospective | Consensus criteria (1992) | 1,101 | All patients in 99 ICUs | 128 | NA | NA |
| Rangel-Frausto et al. 1995 | USA | 01.08.1992-30.04.1993 Prospective | Consensus Criteria (1992) | 3,708 | All adult (>16 yr) patients with SIRS in 3 ICUs and wards | 467 culture positive 527 culture negative | NA | Mean 55 Male 60% All patients with SIRS |
| Brun-Buisson et al. 1995 | France | 04.01.1993-28.02.1993 Prospective | Consensus Criteria + criteria for organ failure | 11,828 | All adult ICU adm 170 ICUs | 1,052 patients 1,064 septic episodes | NA | Mean 61 Male 63% |
| Sands et al. 1997 | USA | 04.01.1993-02.04.1994 Prospective | Consensus criteria (1992) | 12,759 | All adm in ICU, all blood culture-positive in 8 hospitals | 1,166 patients 1,342 septic episodes | 2.8/1,000 patient days | Mean 59 Male 56% |
| Angus et al. 2001 a | USA | 1995 Retrospective | ICD-9 | 6,621,559 | Hospital discharge data, all ages | 192,980 | 3/1,000 population | Mean 59 Male 53% |
| Alberti et al. 2002 | 8 European countries, Canada and Israel | 01.05.1997-31.05.1998 Prospective | Consensus Criteria+ organ failure criteria | 14,364 | All adult (>18 yr) in 28 ICUs | 8,353 (LOS ICU >24 h ) | NA | NA |
| Martin et al. 2003 | USA | 1979-2000 Retrospective | ICD-9 | 750,000,000 | Hospital discharge data, all ages | 10,319,418 patients with sepsis (16.6%-33.6%) | 0.83/1,000 2.4/1,000 population | Mean 57-61 Male 50%-48% |
| Padkin et al. 2003 | England, Wales and Northern Ireland | 01.12.1995-28.02.2000 Retrospective | Prowess criteria | 56,673 | All adult (>16 yr) adm first 24h in 91 ICUs | 15,362 | 0.51/1,000 population | Median 65 Male 54% |
| Flaatten 2004 | Norway | 01.01.1999-31.12.1999 Retrospective | ICD-10 | 700,107 | All hospital patients in 1 year | 2,121 | 1.49/1,000 3/1,000 hospital admission | Mean 58 Male 52% (sepsis) |

| Reference | Country | Time frame and design | Severe sepsis definition | No. of patients screened | Screened population | No. of patients (%) | Incidence | Age (years) Male |
|---|---|---|---|---|---|---|---|---|
| Finfer et al. 2004 | Australia | 01.05.1999-31.07.1999 Prospective | Prowess criteria | 33,543 | All adult (>15 yr) ICU adm 23 ICUs | 691 patients 752 septic episodes | 0.77/1,000 population | Mean 61 Male 57% |
| Brun-Buisson et al. 2004 | France | 19.11.2001-02.12.2001 Prospective | Consensus criteria (1992) | 3,738 | 206 | 546 | 0.95/1,000 population | Median 65 Male 67% |
| Van Gestel et al. 2004 | The Netherlands | 11.12.2001-12.12.2001 Prospective | Prowess criteria | 455 | All adult patients in 47 ICUs | 134 | 0.54/1,000 population | Mean 64 Male 63% |
| Silva et al. 2004 | Brazil | 21.05.2001-31.01.2002 Prospective | Consensus criteria (1992) | 1,383 | All adult ICU adm in 5 ICUs | 241 | 35.6/1,000 patient days | Median 66 Male 59% |
| Sundara-rajan et al. 2005 | Australia | 01.07.1999-30.06.2003 Retrospective | ICD-10 | 3,122,515 | Hospital discharge data, all ages | 13,297 | 0.65/1,000 0.76/1,000 population | NA Male 55% (sepsis) |
| Vincent et al. 2006 | 24 European countries | 01.05.2002-15.05.2002 Prospective | Consensus criteria (1992) | 3,147 | All adult (>15 yr) ICU adm in 198 ICUs | Sepsis 1,177 Severe sepsis 930 | NA | Median 65 Male 63% (sepsis) |
| Harrison et al. 2006 | England, Wales and Northern Ireland | 01.12.1995-31.01.2005 Retrospective | Prowess criteria | 343,860 | All adult (>16 yr) adm in first 24h in 240 ICUs | 92,672 | 0.46-0.66/1,000 population | Mean 60-62 Male 54% (1995 and 2005) |
| Engel et al. 2007 | Germany | One day during 2003 Prospective | Consensus criteria (1992) | 3,887 | All adm in 454 ICUs | 415 | 0.76-1.1/1,000 population | NA |
| Cheng et al. 2007 | China | 01.12.2004-30.11.2005 Prospective | Consensus criteria (1992) | 3,665 | All adult adm in 10 surgical ICUs | 318 | NA | Median 64 65%. |

Age has a strong influence on the incidence of severe sepsis. In the first large retrospective epidemiology study concerning all ages, the incidence was lowest in children aged 5-14 years and in young adults (15-24 years), increasing slowly until the age of 59 years (Angus et al. 2001 a). After 60 years, the incidence increased sharply and was 130 times higher in the elderly over 85 years compared with children. The incidence of severe sepsis in infants (<1 years) was as high as in adult patients aged 60-64 years. Eight percent of sepsis patients were infants, 3.1% aged 1-19 years and 63% were over 60 years in another large retrospective study (Sundarajan et al. 2005). Most incidence studies have included only adult patients (Brun-Buisson et al. 1995; Alberti et al. 2002; Padkin et al. 2003; Vincent et al. 2006; Harrison et al. 2006) but since the incidence in children is low, these studies can be considered representative for the whole population.

The majority of patients in intensive care are male (Reinikainen et al. 2005) and the proportion of men with sepsis or severe sepsis varies from 51.6% to 66.8% (Flaatten 2004; Brun-Buisson et al. 2004). A female majority (51.9%) was found in one study, but in that study, too, men were at increased relative risk (1.28, 95% CI 1.24-1.32) for sepsis (Martin et al. 2003).

Race also influences the incidence of sepsis and severe sepsis. Black people have been found to have higher incidence of sepsis than white people (Martin et al. 2003; Dombrovskiy et al. 2007 a). The incidence is nearly double in black people compared to white people (6.08 vs. 3.58/1,000 population respectively) (Barnato et al. 2008). This difference in incidence remained after adjusting people for poverty or other social factors.

### 2.2.2 Incidence of organ dysfunction

Patients with severe sepsis often have more than one organ dysfunction or failure (Padkin et al. 2003; Guidet et al. 2005; Vincent et al. 2006; Cheng et al. 2007). Organ dysfunction has been defined as SOFA score $\leq 2$ and organ failure as SOFA score $\geq 3$ (Brun-Buisson et al. 2004; Vincent et al. 2006). The most prevalent organ dysfunctions are acute respiratory failure, septic shock and acute renal failure (Angus et al. 2001 a, van Gestel et al. 2004; Vincent et al. 2006). Respiratory dysfunction is usually defined as acute lung injury (ALI) with $P_aO_2/F_iO_2$ relation $\leq$ 300 mmHg ($\leq$ 40 kPa) or acute respiratory distress syndrome (ARDS) with $P_aO_2/F_iO_2$ relation $\leq$ 200 mmHg ($\leq$ 26.7 kPa). Respiratory dysfunction is

found in 50%-96% of patients with severe sepsis (Guidet et al. 2005; Vincent et al. 2006). Septic shock is present in 46%-72% (van Gestel et al. 2004; Engel et al. 2007). Up to 87% of patients with more than one organ failure may have septic shock (Guidet et al. 2005). Acute renal failure is present in 16%-51% depending on the definition of renal failure (serum creatinine value or low urinary output) (Hoste et al. 2003; Vincent et al. 2006). The lower occurrence was found in a retrospective study especially focusing on septic renal failure. Another retrospective study using a new classification for renal failure named RIFLE (Risk, Injury, Failure, Loss and End-Stage Kidney Disease) has found that sepsis is the most frequent precipitating factor (47%) in patients with acute kidney injury (Bellomo et al. 2004).

Haematological dysfunction is usually defined as low platelet count and is present between 12% and 22% of severe sepsis cases (Brun-Buisson et al. 2004; Engel et al. 2007). Sepsis is the most common clinical condition associated with disseminated intravascular coagulation (DIC), the most severe form of haematological dysfunction. In medical and surgical ICU patients with clinical suspicion of DIC, 34% had confirmed diagnosis of DIC according to the International Society for Thrombosis and Haemostasis (ISTH) criteria (Taylor et al. 2001) and 77% of those patients had severe sepsis (Bakhtiari et al. 2004). Hepatic failure is present as a new organ dysfunction only in 0.6%-1.3% of patients with severe sepsis (Sundararajan et al. 2005; Angus et al. 2001 a). Central nervous system dysfunction is most difficult to prove to be of septic origin and the occurrence varies widely from 9% to 30% (Angus et al. 2001 a; Brun-Buisson et al. 2004). Severe sepsis precipitated organ dysfunctions and failures in different studies are presented in Table 4.

**Table 4.** Severe sepsis associated organ dysfunction.

| **Reference and type of the study** | **Definition for organ failure** | **Respiratory** | **Cardio-vascular** | **Renal** | **Haematological** | **Hepatic** | **Central nervous system** |
|---|---|---|---|---|---|---|---|
| Angus et al. 2001 a Retrospective | ICD-9 | 46% | 24% | 22% | 21% | 1% | 9% |
| Hoste et al. 2003 Retrospective | Study specific | 84% | 56% | 16% | 67% | 39% | |
| Brun-Buisson et al. 2004 Prospective | SOFA≥ 3 | 57% | 56% | 21% | 12% | 6% | 30% |
| van Gestel et al. 2004 Prospective | Prowess criteria | 90% | 72% | 53% | 23% | 45% | 23% |
| Guidet et al. 2005 Prospective | ICD-10<br>1 organ failure<br>≥ 2 organ failures | 74%<br>96% | 15%<br>87% | 7%<br>48% | 0.3%<br>4% | | 4%<br>21% |
| Sundararajan et al. 2005 Retrospective | ICD-10 | 23% | 38% | 35% | | 1% | 17% |
| Vincent et al. 2006 Prospective | SOFA ≥3 | 50% | 63% | 51% | 20% | 12% | |
| Engel et al. 2007 Prospective | Modified Prowess criteria | 52% | 46% | 42% | 22% | | 28% |

## 3.2 PATHOPHYSIOLOGY AND MEDIATORS IN SEVERE SEPSIS

*"Severe sepsis and septic shock are clinical manifestations of a dysregulated immune response to invasive pathogens" (Bochud and Calandra 2003).*

Severe sepsis is a result of complex interactions between the infecting microorganism and host's reaction to it. Briefly, the innate immunity must recognise invading microbes locally in a specific tissue or in the systemic circulation. The stimulation of innate immunity will activate intracellular signalling, which will result in the release of various cytokines like tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Cytokines have either proinflammatory or anti-inflammatory properties. Host cells like monocytes, macrophages, neutrophils, dendritic cells and natural killer (NK) cells are activated to eliminate invading pathogens. The innate response also includes complement and coagulant system activation and the production of acute phase proteins. The adaptive immunity consists of humoral and cell-mediated responses to pathogens. Lymphocytes like B cells secrete immunoglobulins to eliminate infecting microorganisms. Cell-mediated immunity includes different types of T cells like T helper and natural killer cells. Some T cells like CD4+T cells can secrete either proinflammatory or anti-inflammatory cytokines depending on the phase of the immune response.

Anti-inflammatory immune suppression may follow the initial cytokine burst seen in sepsis. The proinflammatory response may shift in excess to the anti-inflammatory state. Apoptotic cell death seen in sepsis may cause anergy (loss of cell-mediated immune response) or loss of CD4+T cells, B cells or dendritic cells (Hotchkiss et al. 1999). The central nervous system participates in the immune response by transmitting suppression of proinflammatory cytokine production in macrophages by cholinergic anti-inflammatory pathway mediated via the vagus nerve (Borovikova et al. 2000).

In addition, the polymorphism in TLRs or cytokine producing genes influences the balance between proinflammatory and anti-inflammatory response and hence survival (Freeman and Buchman 2000; Bochud et al. 2008; Wurfel et al. 2008.). Figure 2 presents simplified pathogen–induced immune responses and their outcome.

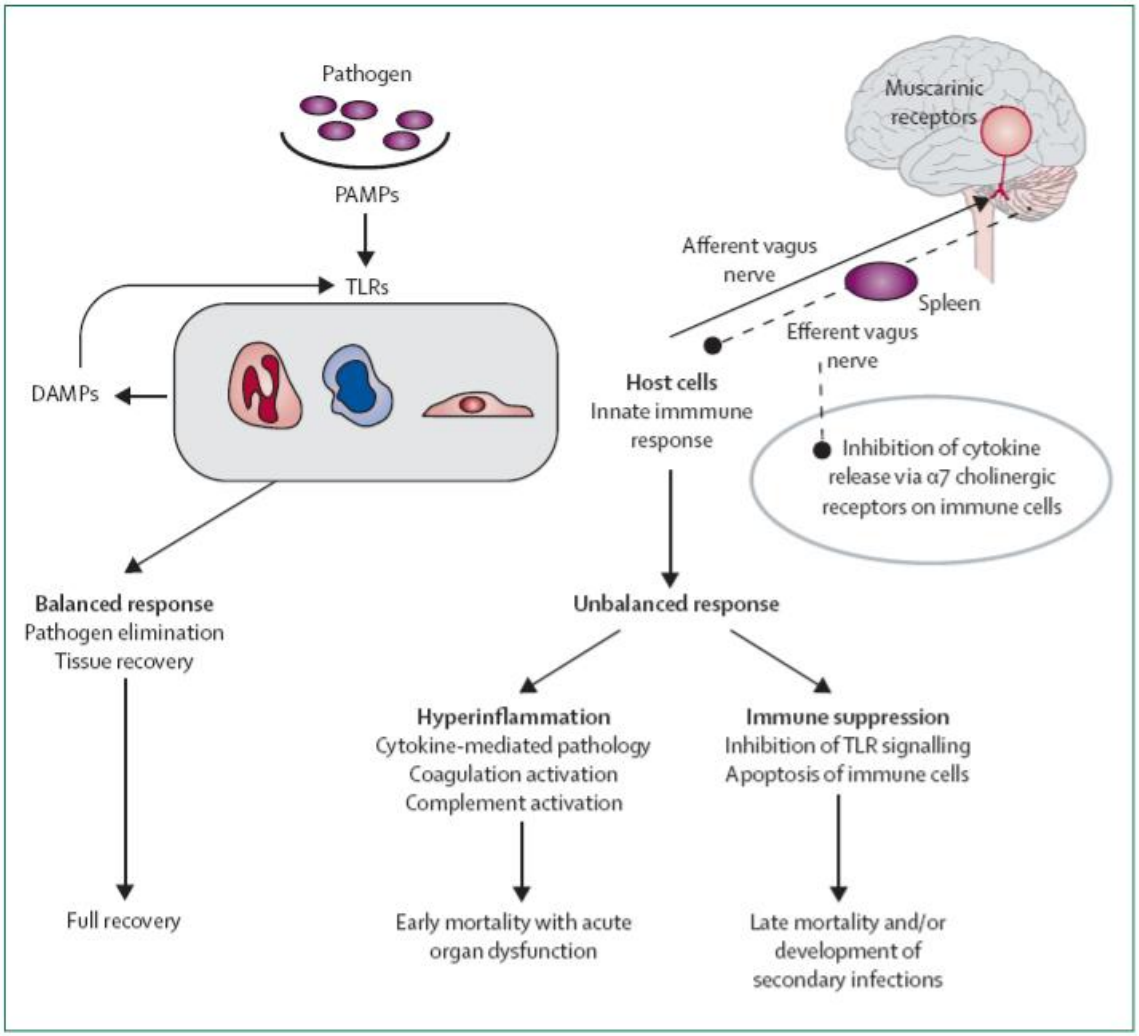



**Figure 2**. Pathogen–induced immune responses in host. Adopted from van der Poll and Opal 2008 with permission.

### 2.3.1 Innate immunity

Innate immunity detects invading pathogens with pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs). PRRs recognise pathogen-associated molecular patterns (PAMPs), which are molecules present in the cell-walls of bacteria, fungi, viruses and parasites. PAMPs are pathogen-specific components like lipopolysaccharide (LPS) in Gram-negative bacteria, lipoteichoic acid in Gram-positive bacteria, peptidoglycan in both Gram-positive and negative bacteria, flagellin in flagellated bacteria, mannan in fungi like Candida albicans and viral RNA. TLRs also recognise molecules able to stimulate the innate immunity response in non-infectious conditions like tissue damage and hypoxia. These molecules are called alarmins (Bianchi 2007) and together with PAMPs they are included in damage-associated molecular patterns (DAMPs) (Bianchi 2007). PAMPs and alarmins seem to have similar effects on PRRs during infectious and non-infectious systemic inflammation. High mobility group box-1 protein (HMGB1) is one of the known alarmins (Yang et al. 2007).

Toll-like receptors (TLRs) are transmembrane proteins present on the surface of immune cells. Ten different TLRs have been found in humans. TLR-4 is specific for LPS and TLR-2 for lipoteichoic acid and peptidoglycan. Intracellular signalling triggers transcription factors like cytosolic nuclear factor κB (NF-κB). Transcription of immune response genes is increased and proinflammatory cytokines like TNF-α and IL-1β are released. NF-κB also stimulates the release of anti-inflammatory cytokine IL-10. Monocytes and macrophages are activated to secrete proinflammatory cytokines. Neutrophils and endothelial cells are activated to produce adhesion molecules, which help in killing pathogens, but may damage endothelium. Macrophages release mediators like vascular endothelial growth factor (VEGF), which increases vascular permeability (Senger et al. 1983) and causes vasodilatation by induction of endothelial nitric oxide synthase (Kroll and Waltenberger 1999).

### 2.3.2 Adaptive immunity

The adaptive immune response consists of humoral immunity like B cells secreting immunoglobulins (antibodies) and cell-mediated immunity like different types of T cells. CD4+T cells are helper cells, which secrete cytokines and CD8+T cells are cytotoxic killer

cells (Delves and Roitt 2000 a). The CD4+helper cells are divided into Th1 and Th2 types depending on the cytokines they produce (Mossman et al. 1986). Th1 type helper cells secrete pro-inflammatory cytokines like TNF-α, IL-2 and interferon-γ (IFN-γ). Th2 type helper cells secrete anti-inflammatory cytokines like IL-4, IL-6, IL-10 and IL-13 (Opal and DePalo 2000). The balance between Th1 and Th2 response is influenced by the dose of the pathogen (Abbas et al. 1996). Antibodies present pathogens to the components of the innate immune system. Pathogens are destroyed by phagocytic cells with the help of the complement system or antibodies binding on the receptors on phagocytic cells (Delves and Roitt 2000 b).

### 2.3.3 Proinflammatory and anti-inflammatory cytokines

Cytokines are soluble protein mediators of the innate and adaptive immune response. Cytokines are synthesized in response to stimulation of immune cells and they usually act locally on adjacent cells, but high concentrations may enter the circulation and have systemic effects. Many cytokines are pleiotrophic, which means that one cytokine can have effects on different cell types. In addition, similar effects can be caused by different cytokines (Tayal and Kalra 2008). TNF-α and IL-1β are main proinflammatory cytokines, which induce the activation of systemic inflammation. Cytokines like IL-4 and IL-10 are solely anti-inflammatory and attenuate the proinflammatory response by decreasing the secretion of certain cytokines or increasing the Th2 response. IL-6 is a pleiotrophic cytokine, which has both proinflammatory and anti-inflammatory properties but the net effect is considered to be anti-inflammatory (Opal and DePalo 2000). Common cytokines, their cellular sources and main actions are listed in Table 5.

**Table 5.** Main proinflammatory and anti-inflammatory cytokines. Modified from references Oberholzer et al. 2000, Delves and Roit 2000 b, Opal and DePalo 2000 and Riedemann et al. 2003.

| Cytokine | Cellular sources | Major activities |
|---|---|---|
| **TNF-α** | Monocytes<br>Macrophages | ***Proinflammatory***<br>-promotes inflammation<br>-activates macrophages and neutrophils<br>-induces the production of adhesion molecules in endothelial cells<br>-induces NO synthase in endothelial cells<br>-activates complement system<br>-activates coagulation |
| **IL-1β** | Monocytes<br>Macrophages | ***Proinflammatory***<br>-promotes inflammation<br>-activates macrophages and T cells<br>-potentiates the effects of TNF-α |
| **IL-1ra** | Monocytes<br>Macrophages<br>Dendritic cells | ***Anti-inflammatory***<br>-IL-1-receptor antagonist (ra)<br>-inhibits IL-1 –mediated cellular activation |
| **IL-2** | T helper cells (Th1) | ***Proinflammatory***<br>-activates lymphocytes, natural killer cells and macrophages |
| **IL-4** | T helper cells (Th2)<br>Mast cells<br>B cells<br>Stromal cells | ***Anti-inflammatory***<br>-inhibits LPS-induced proinflammatory cytokine synthesis<br>-promotes Th2 lymphocyte development |
| **IL-6** | Monocytes<br>Macrophages<br>Endothelial cells<br>Polymorphonuclear cells | ***Proinflammatory***<br>-activates lymphocytes<br>-differentiation of B cells<br>-stimulates the production of acute phase proteins<br>***Anti-inflammatory***<br>-inhibits TNF and IL-1 production by macrophages<br>-stimulates adrenocorticotrophic hormone |
| **IL-8** | Monocytes<br>Macrophages<br>Endothelial cells | ***Proinflammatory***<br>-chemotaxis of neutrophils, basophils and T-cells |
| **IL-10** | T cells (Th2)<br>B cells<br>Monocytes<br>Macrophages | ***Anti-inflammatory***<br>-inhibits monocyte, macrophage and neutrophil cytokine production<br>-inhibits Th1 lymphocyte responses<br>-inhibits IL-2 and IFN-γ<br>-inhibits NF-κB nuclear translocation |
| **IL-11** | Fibroblasts<br>Bone marrow stromal cells | ***Anti-inflammatory***<br>-inhibits monocyte/macrophage proinflammatory cytokine response<br>-promotes Th2 lymphocyte response |
| **IL-13** | T cells (Th2) | ***Anti-inflammatory***<br>-like IL-4<br>-attenuates monocyte and macrophage function |
| **Interferon-γ (IFN-γ)** | T cells (Th1)<br>NK cells | ***Proinflammatory***<br>-activates macrophages<br>inhibits Th2 lymphocyte responses |
| **HMGB1** | Macrophages<br>Dendritic cells<br>Natural killer cells<br>Necrotic cells | ***Proinflammatory***<br>-stimulates monocytes to produce TNF-α, IL-1β, IL-6<br>-regulates fibrinolysis by secreting PAI-1 and tPA |
| **Macrophage migration inhibitory factor (MIF)** | Monocytes<br>Macrophages<br>T cells, B cells<br>Epithelial cells | ***Proinflammatory***<br>-activates T cells<br>-stimulates macrophages<br>-modulates the expression of TLR4 on macrophages |

### 2.3.4 High mobility group box-1 protein

The data showing that high HMGB1 is associated with the severity of sepsis are mainly from animal studies (Wang et al. 1999). Studies in humans have shown conflicting results. HMGB1 has been associated with mortality in patients with severe sepsis (Wang et al. 1999) but in other patients with severe sepsis there was no correlation between HMGB1 and outcome (Sunden-Cullberg et al. 2005). Study results have not been reproduced even in a specific group of patients such as patients with community-acquired pneumonia and severe sepsis. A Danish study showed no associated mortality with HMGB1 concentrations (Gaïni et al. 2007) while the other study in the United States showed a correlation (Angus et al. 2007).

In experimental animal studies HMGB1 concentrations have been associated with organ failures such as shock (Wang et al. 1999) and acute lung injury (Abraham et al. 2000). HMGB1 concentrations have not been associated with organ dysfunctions in many other studies, either (Sunden-Cullberg et al. 2005; Angus et al. 2007; van Zoelen et al. 2007). HMGB1 concentrations were elevated in one study investigating patients with DIC and high HMGB1 correlated with organ dysfunction and even with mortality (Hatada et al. 2005). However, only 20% of these patients had DIC associated with infection.

### 2.3.5 Vascular endothelial growth factor

VEGF has been studied extensively in various diseases like cardiovascular diseases, malignancies, ocular neovascularisation, pre-eclampsia and inflammatory diseases such as rheumatoid arthritis but there are only few human studies measuring VEGF in patients with severe sepsis (Pickers et al. 2005; van der Flier et al. 2005). These studies have found that high VEGF correlated with the severity of organ dysfunction and mortality. High plasma VEGF has been correlated with ARDS (Thickett et al. 2001) while intrapulmonary VEGF has been reduced in these patients (Thickett et al. 2002).

VEGF antagonist, a soluble VEGF receptor 1 (fms-like tyrosine kinase 1, sFLT) has been found to inhibit the effects of VEGF (Maynard et al. 2003) and recent studies have measured VEGF together with sFLT both in experimental (Tsao et al. 2007) and human studies (Shapiro et al. 2008). In the latter study, patients with septic shock had increasing

concentrations of VEGF and sFLT compared to the patients without shock and particularly high sFLT correlated with APACHE II and SOFA scores. In the future, sFLT may have diagnostic and even therapeutic applications (Shapiro et al. 2008).

### 2.3.6 Complement activation

The complement system is activated as a soluble part of innate immunity. The classic pathway is precipitated by the component C1q, which is able to bind to immunoglobulins. The alternative pathway is triggered after exposure to surface molecules containing carbohydrates and lipids (i.e. LPS). The cleavage product of C3, the C3b, enhances phagosytosis by binding to the pathogen (Delves and Roitt 2000 a). As a result of the activation of any of these pathways, the concentrations of C3a, C4a and C5a are increased. C5a is a strong chemoattractant and recruits inflammatory cells to produce cytokines and activates phagocytic cells (Guo and Ward 2005). In addition, C5a may increase the apoptosis of thymocytes and hence diminish the anti-inflammatory response (Guo et al. 2000).

### 2.3.7 Coagulation activation

The procoagulant system is activated by the innate immune response and as a consequence the common procoagulant-anticoagulant balance is disturbed. Inflammation and coagulation are linked with tissue factor (TF) pathway, thrombin, the protein C system and the fibrinolytic system (Levi and van der Poll 2005). Proinflammatory cytokines, particularly IL-6, provoke endothelial cells to expose TF and activated monocytes to secrete TF. Coagulant cascade in sepsis is activated by TF and results in the formation of thrombin and fibrin clots in microvasculature (Thijs et al. 1993).

### 2.3.8 Immune suppression and apoptosis

Immune response in sepsis may be impaired by excess immunosuppression caused by anergy (unresponsiveness to antigen stimulation) of the immune cells or apoptosis (programmed cell death) of immune cells (Hotchkiss et al. 1999). Anergy of T cell proliferation and depressed production of IL-2 and TNF-α have been found in patients with lethal intra-abdominal sepsis (Heidecke et al. 1999).

Extensive lymphocyte apoptosis have been shown to occur in both animal (Hiramatsu et al. 1997) and human sepsis studies (Hotchkiss et al. 1999). Apoptosis is initiated by caspases (Budihardjo et al. 1999) and glucocorticoids are able to induce it (Fukuzuka et al. 2000). CD4+T cells, B cells and also follicular dendritic cells are the most abundant cells to die (Hotchkiss et al. 2001; Tinsley et al. 2003), but macrophages do not suffer from apoptosis (Hotchkiss et al. 2002). In addition, apoptotic cells induce monocytes to secrete anti-inflammatory cytokines like IL-10 and decrease the secretion of proinflammatory cytokines like TNF-α and IL-1 (Voll et al. 1997). Unlike apoptotic cells, necrotic cells induce inflammatory response (Green and Beere 2000).

### 2.3.9 Cholinergic anti-inflammatory pathway

The central nervous system may influence the innate immune response via the efferent parasympathetic anti-inflammatory pathway (Borovikova et al. 2000). A nicotinic acetylcholine receptor alpha7 (α7) subunit transmits the acetylcholine effect on macrophages and its stimulation inhibits the TNF release (Wang et al. 2003). Treatment with nicotine, an α7 agonist, has also been reported to reduce HMGB1 release on macrophages in experimental models of sepsis (Wang et al. 2004). Oxytocin, a hormone with neurotransmitter properties, has excitatory effects on vagal neurons and was recently shown to decrease cytokine response to bacterial endotoxin in healthy men (Clodi et al. 2008).

## 2.4 TREATMENT OF SEVERE SEPSIS

### 2.4.1 Fluid therapy

Fluid resuscitation is essential in treating sepsis-induced cardiovascular failure and septic shock. Absolute and relative hypovolaemia are caused by external and internal fluid losses (sweating, diarrhoea, fluid shifts to the peritoneal cavity) and the maldistribution of circulation due to vasodilatation. Hypovolaemia may lead to reduced cardiac output, low blood pressure and disturbances in microcirculation. The time for fluid resuscitation, the adequate amount of fluids and fluid treatment protocols with defined endpoints seem to be more important for good outcome than the type of fluids (Rivers et al. 2001).

*The type of fluids*

Meta-analyses concerning fluid resuscitation in general ICU population show no differences in outcome between crystalloids and colloids (Choi et al. 1999; Perel et al. 2007). However, although there are no safety or efficacy differences in fluid resuscitation between colloids (albumin, gelatins and hydroxyethyl starches) in general (Bunn et al. 2008), there are safety concerns regarding in patients with severe sepsis. The risk for acute kidney injury (AKI) with hydroxyethyl starches (HES) but not gelatines has been found to increase (Schortgen et al. 2001). The acute renal failure and need for continuous renal replacement therapy with 10% high molecular weight HES solutions (200/0.5) increased in patients with severe sepsis (Brunkhorst et al. 2008) and hyperoncotic colloids are not recommended because of possible risk of renal injury in patients with shock for any cause (Schortgen et al. 2008). Albumin has been suspected to increase mortality in critically ill patients (Cochrane Injuries Group Albumin Reviewers 1998) but was found to be safe compared with saline in the SAFE Study (Finfer et al. 2004 b). The mortality rates did not differ in a subgroup of patients with severe sepsis, either. In Finland, albumin is not recommended instead of crystalloids or other colloids (Pettilä and Ruokonen 2005).

*The fluid challenge*

The concept of fluid challenge is based on the patient's response to fluids administered (Weil et al. 1979). The use of fluid challenge is not limited to haemodynamically unstable septic patients but can be used in any patient group with hypovolaemia. The fluid challenge protocol should include the type of fluid, the rate of fluid administration, the

endpoints and safety limits (Vincent and Weil 2006). As discussed earlier, the type of fluid can be chosen quite freely. The proposed rate of fluid administration has varied from 50-200 ml in 10 minutes to 500-1000 ml of crystalloids or 300-500 ml of colloids in 30 minutes (Weil et al. 1979; Dellinger et al. 2008). The main target in fluid challenge should be haemodynamic improvement. However, the precise end points for fluid challenge or resuscitation are not self-evident or universally accepted. Mean arterial pressure (MAP), cardiac index (CI), cardiac filling pressures such as central venous (CVP) and pulmonary artery wedge (pcwp) pressures, mixed venous saturation (SvO2) or central venous saturation (ScvO2) and stroke volume variation (SVV) (Marx et al. 2004) have been used as end points.

The CVP has been chosen for one of the fluid resuscitation goals in severe sepsis treatment guidelines (Dellinger et al. 2008), but cardiac filling pressures have been shown not to predict hemodynamic response to fluid challenge (Osman et al. 2007). SVV does not work at least on pressure support ventilation in patients with septic shock (Perner et al. 2006). The individual safety limits for fluid challenge technique are needed to avoid congestive heart failure and pulmonary oedema (Vincent and Weil 2006). The fluid challenge technique should be used especially in early treatment of haemodynamically unstable patients and once the need for additional fluids has passed, the fluid administration should be decreased. Excessive fluids can be harmful in patients with acute lung injury. Patients with restricted fluids are weaned earlier from the ventilator and have shorter length of stay in the ICU than patients with liberal fluid management (Sakr et al. 2006).

### 2.4.2. Vasoactive treatment

*The target for vasoactive treatment*

Severe sepsis and septic shock as the most severe form of cardiovascular dysfunction cause absolute or relative hypovolaemia, vasodilatation, direct myocardial depression and consequently hypotension, changes in blood flow distribution and perfusion abnormalities. Fluid resuscitation alone can alleviate haemodynamic disturbances only in a mild cardiovascular dysfunction and patients with septic shock need vasoactive support in 85% of cases (Annane et al. 2003). Mean arterial pressure (MAP) over 65 mmHg has been recommended by the Surviving Sepsis Campaign guidelines (Dellinger et al. 2008). The optimal target level for MAP is not known and it may vary between individual patients

with different pre-existing comorbidities. MAP under 65 mmHg may impair outcome (Varpula et al. 2005), but high MAP up to 90 mm Hg with rapidly increasing systemic vascular resistance increases mortality as well (Lopez et al. 2004). However, increasing MAP from 65 mm Hg to 85 mm Hg with NE in a small group of patients with septic shock did not increase or decrease the systemic oxygen metabolism, diuresis or splanchnic perfusion (LeDoux et al. 2000).

Myocardial depression has been found in 44-50% in patients with septic shock (Parker et al. 1984; Charpentier et al. 2004). Survivors usually recover completely, but myocardial adrenergic hyporesponsiveness found in septic shock can persist for several days, resolving in 8-10 days (Parker et al. 1984; Cariou et al. 2008). The target for inotropic treatment is to increase the cardiac output (CO) and oxygen delivery (DO2). Low SvO2 or ScvO2 can be used as markers of inadequate oxygen delivery, but the latter corresponds poorly in patients with severe sepsis (Varpula et al. 2006). Increasing oxygen delivery to supranormal values does not decrease mortality in patients with critical illness (Hayes et al. 1993; Gattinoni et al. 1995). In the former study 40% of the patients included had severe sepsis or septic shock, in the latter study the proportion of septic patients cannot be identified. The SSC guidelines do not recommend increasing the CI to supranormal levels in patients with severe sepsis, either (Dellinger et al. 2008).

*Vasopressor drugs*

Dopamine is a precursor of norepinephrine and epinephrine. Its effects are dose-dependent: stimulation of vasodilatory dopaminergic DA1 receptors in renal and mesenteric vessels at low doses (<5 μg/kg/min), β-adrenergic stimulation with increasing contractility and heart rate at 5-10 μg/kg/min and α-adrenergic stimulation with vasoconstriction at high doses >10 μg/kg/min. However, dopamine does not prevent acute renal failure (Bellomo et al. 2000). Dopamine can increase splanchnic blood flow, but decreases the mucosal flow and hence oxygen delivery (Giraud et al. 1984). Dopamine can have different unwanted endocrinological effects such as suppressing prolactin release (Bailey et al. 1997) and impairing growth hormone release (van den Berghe et al. 1994). Dopamine administration may increase mortality compared with patients not treated with dopamine for septic shock treatment (Sakr et al. 2006).

Norepinephrine (NE) is an α-adrenergic agonist with weak β-adrenergic agonist effects. It is a potent vasopressor which can be effective in dopamine-resistant septic shock (Martin

et al. 1994; de Backer et al. 2003). The effect of NE on splanchnic blood flow and oxygen delivery has been found to be similar to that of dopamine in moderate septic shock but more advantageous than epinephrine in severe septic shock (de Backer et al. 2003). While increasing systemic oxygen delivery, NE also increases mucosal pH, a surrogate marker of tissue oxygenation. NE appears to have a favourable effect on the balance between splanchnic oxygen delivery and oxygen utilization (Marik et al. 1994). In all, the influence of dopamine or NE on regional blood flow cannot be predicted from changes in systemic blood flow and the increased oxygen demand may lead to increased risk for tissue hypoxia in the splanchnic region (Ruokonen et al. 1993). NE and dopamine are the first line vasopressors recommended by the SSC guidelines.

Epinephrine is a strong α-adrenergic and β-adrenergic agonist. Epinephrine increases blood pressure and cardiac index in patients with septic shock in a highly dose dependent manner (Moran et al. 1993) but it impairs the splanchnic blood flow compared with the combination of NE and dobutamine (Levy et al. 1997) or NE alone (de Backer et al. 2003). Epinephrine is not recommended as a first line vasopressor agent in septic shock (Dellinger et al. 2008). However, one recent study found no difference in mortality in patients with septic shock treated with epinephrine alone or with NE combined with dobutamine (Annane et al. 2007).

Arginine vasopressin (AVP) is an endogenous peptide hormone synthesized in the hypothalamus and secreted by the pituitary gland. It directly activates V1 receptors in vascular smooth muscle cells leading to vasoconstriction and increases the vascular responsiveness to catecholamines (Medina et al. 1997). AVP may also inhibit the smooth muscle nitric oxide production in inflammatory states (Kusano et al. 1997). The activation of V2 receptors on renal tubules and water resorption causes the antidiuretic effect of the hormone. V3 receptors are situated in the pituitary gland and have central effects, such as ACTH release. In hypotensive states, AVP should be maintaining the blood pressure, but AVP plasma levels have been found to be inadequately low in septic shock (Landry et al. 1997). Low doses of AVP infusion increases arterial blood pressure alone or in combination with NE even in catecholamine–resistant vasodilatory shock (Landry et al. 1997; Dünser et al. 2003 a). Vasopressin should be used as a low-dose infusion (between 0.01 and 0.04 U/min) to prevent harmful side effects (Beale et al. 2004). As a potent vasoconstrictor, AVP may be harmful and may cause a decrease in CO and even cardiac

arrest (Holmes et al. 2001), mesenterial ischaemia (van Haren et al. 2003. Klinzing et al. 2003) and ischaemic skin lesions (Dünser et al. 2003 b). AVP may also induce thrombocytopenia by platelet aggregation (Dünser et al. 2004). A recent multicentre, randomized, double-blind trial found no difference in mortality or side effects in patients with moderate or severe septic shock treated with NE or low-dose AVP (max 0.03 U/min) (Russell et al. 2008). Patients with severe ischaemic heart disease or mesenteric ischaemia were excluded from the study and this may have influenced the safety profile of AVP. Surprisingly, there was a trend for a lower mortality in patients with less severe septic shock treated with AVP. Low dose AVP may be more advantageous in replenishing AVP reserves than in just using it as a high-dose vasoconstrictor alone.

Terlipressin is a long-acting synthetic vasopressin analogue, which has higher V1 receptor activity than AVP. Terlipressin been used as a bolus injection to stabilize haemodynamics in septic shock (Leone et al. 2004; Morelli et al. 2004). Terlipressin reduces CI, DO and oxygen consumption and may carry a risk for sustained global and regional vasoconstriction (Albanese et al. 2005). It should possibly be given together with an inotrope like dobutamine to maintain CI (Morelli et al. 2008). Neither AVP nor terlipressin are recommended by the SSC guidelines as first line agents in adult septic shock (Dellinger et al. 2008).

*Inotropic drugs*

Dobutamine, a synthetic inotrope has a direct effect on β1 cardiac receptors and only a slight effect on α and β2 vascular receptors (Tuttle et al. 1975). Dobutamine was first compared with dopamine in patients with septic shock and was recommended in cases with high filling pressures and cardiac failure (Regnier et al. 1979; Jardin et al. 1981). Since then it has been widely used to increase CO and DO in patients with severe sepsis and septic shock (Shoemaker et al. 1986; Vincent et al. 1990). Dobutamine was even able to increase DO to supranormal levels (Shoemaker et al. 1988; Tuchschmidt et al. 1992), but as pointed out earlier, this was not found to be advantageous (Hayes et al. 1993; Gattinoni 1995). Dobutamine is the first line inotrope to be used in septic shock (Dellinger et al. 2008).

Levosimendan, a calcium-sensitizer with inotropic and vasodilative properties has been shown to be effective in acute and chronic heart failure (Slawsky et al. 2000; Follath et al. 2002) even if it has not affected mortality compared with dobutamine (Mebazaa et al.

2007). Levosimendan increases CI in sepsis-induced myocardial depression both in experimental sepsis (Oldner et al. 2001) and in patients with septic shock (Morelli et al. 2005). Levosimendan alleviates right ventricular dysfunction in patients with septic shock-associated acute respiratory distress syndrome (ARDS) (Morelli et al. 2006). Levosimendan increases intestinal blood flow and gut oxygen delivery in experimental endotoxemia (Oldner et al. 2001; Schwarte et al. 2005; Dubin et al. 2007) and increased gastric mucosal blood flow has been shown in patients with septic shock (Morelli et al. 2005). Even though levosimendan has many advantageous effects in sepsis-induced myocardial depression, there are as yet no studies to show the reduction in mortality compared with dobutamine.

### 2.4.3 Early goal-directed therapy

Fluid resuscitation and vasoactive therapy have been the cornerstones of haemodynamic treatment in severe sepsis for nearly two decades (Shoemaker et al. 1991) but the concept of early treatment with several strict predefined goals was introduced by Rivers et al. 2001. This early goal-directed therapy (EGDT), which was conducted mainly in the emergency department in the first six hours, had targets for MAP, CVP, urine output and ScvO2. Fluid resuscitation, vasopressor therapy, dobutamine and packed red blood cells were the treatments of choice to achieve predefined targets. The reduction in mortality was 16% in the goal-directed group compared with the control group. This study has been criticized for its patient selection and for being only a single-centre study (Ho et al. 2006), but it was the first study to show a mortality reduction using a protocol with goals for basic haemodynamic variables. The EGDT concept has been widely used after publication and decreased mortality after implementation of EGDT protocol has been documented in various hospitals (Gao et al. 2005; Micek et al. 2006; Jones et al. 2007).

### 2.4.4 Antimicrobial treatment

As a disease of infectious origin, severe sepsis needs to be treated with antimicrobial agents as well as adequate source control whenever possible. Experimental studies in septic shock have shown that haemodynamic treatments need to be combined with antimicrobial agents and vice versa for better survival (Natanson et al. 1990). Appropriate empirical antimicrobial treatment has been shown to decrease mortality in blood-born infections (Leibovici et al. 1998; Ibrahim et al. 2000). Treatment should be started with broad-spectrum antibiotics, which can later be matched up with the causative agent. The timing of antimicrobial treatment is as important as effectiveness and should be initiated as soon as possible. Early antimicrobial treatment administered within on hour after documented hypotension in patients with septic shock has been shown to increase survival compared with later administration. One-hour's delay in antibiotic administration decreases the probability of survival by 12% in septic shock (Kumar et al. 2006).

### 2.4.5 Mechanical ventilation

Respiratory failure is the most common organ dysfunction in severe sepsis affecting as many as 74%-96% of patients (Guidet et al. 2005). In studies on ALI or ARDS 32%-83% of patients were septic (Amato et al. 1998; Bersten et al. 2002). The first study ever to show decreased mortality with a special method of mechanical ventilation was the ARDSnet Study, and in that study nearly a third of patients had sepsis-related respiratory failure. The six months mortality decreased from 39.8% to 31% in patients ventilated with low tidal volumes ($\leq$ 6 ml/kg predicted body weight) compared with traditional ventilation (The Acute Respiratory Distress Syndrome Network 2000). These patients were also treated with plateau pressures under 30 cm of water. However, higher plateau pressures (up to 40 cm of water) were not associated with increased mortality or complications when used with low tidal volume ventilation (Meade et al. 2008). Nearly half of these patients had sepsis-originated ALI or ARDS. Patients with severe sepsis are treated with a low tidal volume protocol, because at the moment there is no evidence that septic lung injury should be treated differently from other lung injuries.

### 2.4.6 Renal replacement therapy

Severe sepsis is complicated up to 42%-53% by acute kidney injury (AKI) (van Gestel et al. 2004; Bagshaw et al. 2008) and as many as 70% of those patients may need renal replacement therapies (Hoste et al. 2003). In studies on AKI incidence or different treatment modalities 35%-63% of patients were septic (Brivet et al. 1996; Vinsonneau et al. 2006; Prescott et al. 2007). Even though two meta-analyses and one controlled study found no differences in mortality with continuous renal replacement therapies or intermittent haemodialysis in acute renal failure (Kellum et al. 2002; Tonelli et al. 2002; Vinsonneau et al. 2006), continuous treatments are better tolerated and facilitate better fluid balance control in patients with unstable haemodynamics and septic shock. The higher dosage of renal replacement therapy decreased mortality in studies using intermittent haemodialysis (Schiffl et al. 2002) or continuous veno-venous haemofiltration (Ronco et al. 2000). In the former study 36% and in the latter study 13% of the study patients were septic. However, a recent prospective study could not confirm this positive dosage effect on mortality or recovery of kidney function. In this large study, over 50% of study patients had severe sepsis-related acute kidney injury (VA/NIH Acute Renal Failure Trial network 2008). High volume haemofiltration has been shown to remove cytokines (Bellomo et al. 1993), attenuate vasopressor requirements and even have a trend towards decreased mortality in septic shock (Oudemans-van Straaten 1999; Cole et al. 2001; Ratanarat et al. 2005), but prospective, randomized, controlled trials are lacking.

### 2.4.7 Adjuvant treatments

*Glucose control*

Intensive insulin treatment with strict glycaemic control was introduced and widely accepted after a study showing a morbidity and mortality reduction in critically ill surgical patients (van den Berghe et al. 2001). Patients were mostly (63%) postoperative cardiac patients. Mortality was decreased from 8% in patients with blood glucose levels between 10.0-11.1 mmol/l to 4.6% in patients with strict blood glucose levels between 4.4-6.1 mmol/l. The reduction in morbidity was most obvious in bloodstream infections and acute renal failure and the reduction in mortality due to severe sepsis and multiple organ failure was most prominent. This study has been criticized for patient selection, for high mortality in the control group and high glucose administration (Brunkhorst et al. 2008). A second

study in medical critically ill patients failed to show any mortality reduction (van den Berghe et al. 2006). In that study it was impossible to identify the proportion of patients with severe sepsis. A recent large multicentre study on patients with severe sepsis found no benefit from strict glucose control (Brunkhorst et al. 2008). The study was preterminated because of risk of potentially harmful hypoglycaemia (<2.2 mmol/l) in the strict protocol group (17% vs. 4.1% in the control group). The control of the blood glucose level seems to be more important than the absolute levels of exogenous insulin infused and the possible mortality benefit may be greatest below a predicted glucose level of 8.0-11.1 mmol/l (Finney et al. 2003). Target glucose level under 8.3 mmol/l may have the best risk-benefit ratio, probably also in patients with severe sepsis. A large multi-centre, randomised open label glucose control study with targeted 6,000 patients is ongoing and will hopefully demonstrate the appropriate glucose level with maximal advantages and minimal risks (http://www.controlled-trials.com/ISRCTN049682759. NICE-SUGAR study). In addition, it is clinically important to pay attention to bedside glucose measurement, as point-of-care glucometers may measure incorrectly high glucose values in the presence of low hematocrit (Mann et al. 2008).

*Corticosteroids*

High dose corticosteroids (e.g. methylprednisolone 30 mg/kg intravenously) were studied in treating severe sepsis or septic shock in the 1980´s after positive findings in experimental animal studies and one small human study (Schumer 1976). Large, randomised studies could not show any reduction in mortality and in fact high dose corticosteroids were found to be harmful (Sprung et al. 1984; Bone et al. 1987; The Veterans Administration Systemic Sepsis Cooperative Study Group 1987; Luce et al. 1988). Meta-analyses later confirmed these findings and concluded that high dose corticosteroids should not be used in severe sepsis and septic shock (Cronin et al. 1995; Minneci et al. 2004).

Low dose corticosteroid therapy was introduced after it was found that relative adrenal insufficiency in patients with septic shock could lead to vasopressor-resistant hypotension and increased mortality (Rothwell et al. 1991; Soni et al. 1995). Increased survival and better shock reversal were achieved by low dose corticosteroids in prospective, single-centre studies (Bollaert et al. 1998; Briegel et al. 1999; Keh et al. 2003). As high dose corticosteroids were usually administered in the first 24 hours in older studies, low dose

corticosteroid treatment (e.g. hydrocortisone 200-300 mg per day intravenously) was administered over 5 days.

The first prospective, multicentre study was published 2002. In that study by Annane, 77% of patients with septic shock were non-responders to adrenocorticotropic hormone (ACTH) stimulation test and suffered from relative adrenal insufficiency. Low dose corticosteroid treatment with hydrocortisone 50 mg every 6 hours and fludrocortisone 50 μg every 24 hours for 7 days decreased mortality in patients with adrenal insufficiency and shortened the time for shock reversal (Annane et al. 2002). The result of this study was quickly and widely accepted. However, an adequate diagnosis of relative adrenal insufficiency in critically ill patients has been difficult to obtain (Loisa et al. 2005; Lipiner-Friedman et al. 2007; Sprung et al. 2008). Free cortisol levels have been measured instead of total cortisol concentrations, but these have not been helpful in diagnosing relative adrenal insufficiency (Hamrahian et al. 2004; Bendel et al. 2008).

The study by Annane was criticized for the statistical significance of 28-day mortality reduction in non-respondent group and high proportion of patients with earlier exposure to etomidate, a known suppressor of adrenal function (Wagner et al. 1984). A recent large multicentre trial (CORTICUS) failed to show any reduction in mortality in patients with septic shock, whether they were non-responders to the ACTH test or not, and whether they received hydrocortisone or not. Hydrocortisone treatment hastened shock reversal, but was associated with more episodes of superinfection (Sprung et al. 2008). However, as the patients in Annane's study were sicker than the patients in the Corticus Study at randomisation, those patients with vasopressor-unresponsive shock may have benefited from early low dose corticosteroid treatment (Sprung et al. 2008). A new term "critical illness–related corticosteroid insufficiency" (CIRCI) is now introduced. CIRCI is based on more clinical suspicion and judgement than laboratory values in patients with vasopressor-dependent septic shock (Marik et al. 2008).

*Activated protein C*

Inflammation and sepsis can downregulate fibrinolytic and protein C anticoagulant pathways, (Esmon et al. 1999) and recombinant human activated protein C (rhAPC) has been shown to reduce mortality in patients with severe sepsis in the large, randomised, multicentre Prowess Study (Bernard et al. 2001). The majority of patients (76%) in the

Prowess trial had two or more organ dysfunctions and the mean APACHE II score was 25 (SD 7.8). The absolute mortality reduction was 6.1% and relative mortality reduction 19.4% in rhAPC-treated patients. The anti-inflammatory and fibrinolytic effects were demonstrated by decreased IL-6 and fibrin degradation products (D-dimer) levels in patients treated with rhAPC. As a physiological anticoagulant, rhAPC was associated with serious bleeding complications in 3.2% vs. 2% in the control group. Other studies with rhAPC in adult patients with lower risk of death or in children have failed to show any mortality reduction (Abraham et al. 2005; Nadel et al. 2007) and instead have been associated with increased risk of serious bleeding complications (Abraham et al. 2005; Goldstein et al. 2006). A recent Cochrane Database review found "no evidence suggesting that APC should be used for treating patients with severe sepsis or septic shock, unless additional randomised controlled trials provide evidence of a treatment effect" (Martí-Carvajal et al. 2008).

### 2.4.8 Protocols and guidelines

The Surviving Sepsis Campaign has published the Resuscitation Bundle for the first 6 hours and the Management Bundle for the first 24 hours after hospital admission and the diagnosis of severe sepsis (http://www.survivingsepsis.org/implement/bundles). These bundles are presented in Table 6. Mortality in severe sepsis has decreased when these SSC guidelines or modified protocols have been implemented in a few units (Gao et al. 2005; Kortgen et al. 2006) or nationwide (Ferrer et al. 2008). The Spanish study was able to show prospectively how better compliance with the bundles decreased mortality (Ferrer et al. 2008). One year after the study, the compliance with the resuscitation bundle had declined, but compliance with the management bundle and the effect on mortality remained unchanged.

**Table 6.** Resuscitation Bundle for the first 6 hours and the Management Bundle for the first 24 hours. Adopted from the Severe Sepsis Campaign with permission.

**SEPSIS RESUSCITATION BUNDLE**

*The goal is to perform all indicated tasks 100%of the time within the first 6 hours of identification of severe sepsis.*

*The tasks are:*

1. Measure serum lactate
2. Obtain blood cultures prior to antibiotic administration
3. Administer broad-spectrum antibiotic, *within 3 hrs of ED admission and within 1 hour of non-ED admission*
4. In the event of hypotension and/or a serum lactate > 4 mmol/L
    a. Deliver an initial minimum of 20 ml/kg of crystalloid or an equivalent
    b. Apply vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure (MAP) > 65 mm Hg
5. In the event of persistent hypotension despite fluid resuscitation (septic shock) and/or lactate > 4 mmol/L
    a. Achieve a central venous pressure (CVP) of ≥ 8 mm Hg
    b. Achieve a central venous oxygen saturation (ScvO2) ≥ 70 % or mixed venous oxygen saturation (SvO2) ≥ 65 %

**SEPSIS MANAGEMENT BUNDLE**

*Efforts to accomplish these goals should begin immediately, but these items may be completed within 24 hours of presentation for patients with severe sepsis or septic shock.*

1. Administer low-dose steroids for septic shock in accordance with a standardized ICU policy. *If not administered*, document why the patient did not qualify for low-dose steroids based upon the standardized protocol.
2. Administer drotrecogin alfa (activated) in accordance with a standardized ICU policy. *If not administered*, document why the patient did not qualify for drotrecogin alfa (activated).
3. Maintain glucose control ≥ 70, but < 150 mg/dl
4. Maintain a median inspiratory plateau pressure (IPP)* < 30 cm H2O for mechanically ventilated patients

***For questions or concerns, please contact the Critical Care Fellow On-Call.***

Cooper
University Hospital

## 2.5 OUTCOME OF SEVERE SEPSIS

### 2.5.1 Short-term mortality

Severe sepsis is associated with high short-term mortality, ranging from 27% to 59% (Brun-Buisson et al. 1995; Flaatten et al. 2004). ICU and hospital mortalities are representative of the short term outcome and ICU and hospital mortalities for severe sepsis are presented in Table 7.

**Table 7.** Short-term mortality in severe sepsis. NA=not applicable.

| Reference | Country | ICU mortality | Hospital mortality |
|---|---|---|---|
| Salvo et al. 1995 | Italy | NA | 52% |
| Rangel-Frausto et al. 1995 | USA | NA | 20% |
| Brun-Buisson et al. 1995 | France | 56% | 59% |
| Sands et al. 1997 | USA | NA | 34% (28 days) |
| Angus et al. 2001 a | USA | NA | 28.6% |
| Martin et al. 2003 | USA | NA | 27.8%-<br>17.9% in sepsis |
| Padkin et al. 2003 | England, Wales and Northern Ireland | 35% | 47% |
| Flaatten 2004 | Norway | NA | 27% |
| Finfer et al. 2006 | Australia | 26.5% | 37.5% |
| Brun-Buisson et al. 2004 | France | NA | 35% (30 days)<br>41.9% (2 months) |
| Silva et al. 2004 | Brazil | 21.8% | 46.9% |
| Sundarajan et al. 2005 | Australia | NA | 31.1% |
| Vincent et al. 2006 | 24 European countries | 32.2% | NA |
| Engel et al. 2007 | Germany | 48.4% | 55.2% |
| Cheng et al. 2007 | China | NA | 48.7% |
| Dombrovskiy et al. 2007 b | USA | NA | 45.8% (1993)<br>37.8% (2003) |

Case fatality has decreased over time, even though overall severe sepsis mortality accumulates due to the annual incidence increase as shown in Figure 3 (Martin et al. 2003;

Dombrovskiy et al. 2007 b). Mortality is affected by age, gender, race, co-morbidities, the number of organ dysfunctions and underlying genetic factors, as well as the ICU performance in treating patients with severe sepsis.

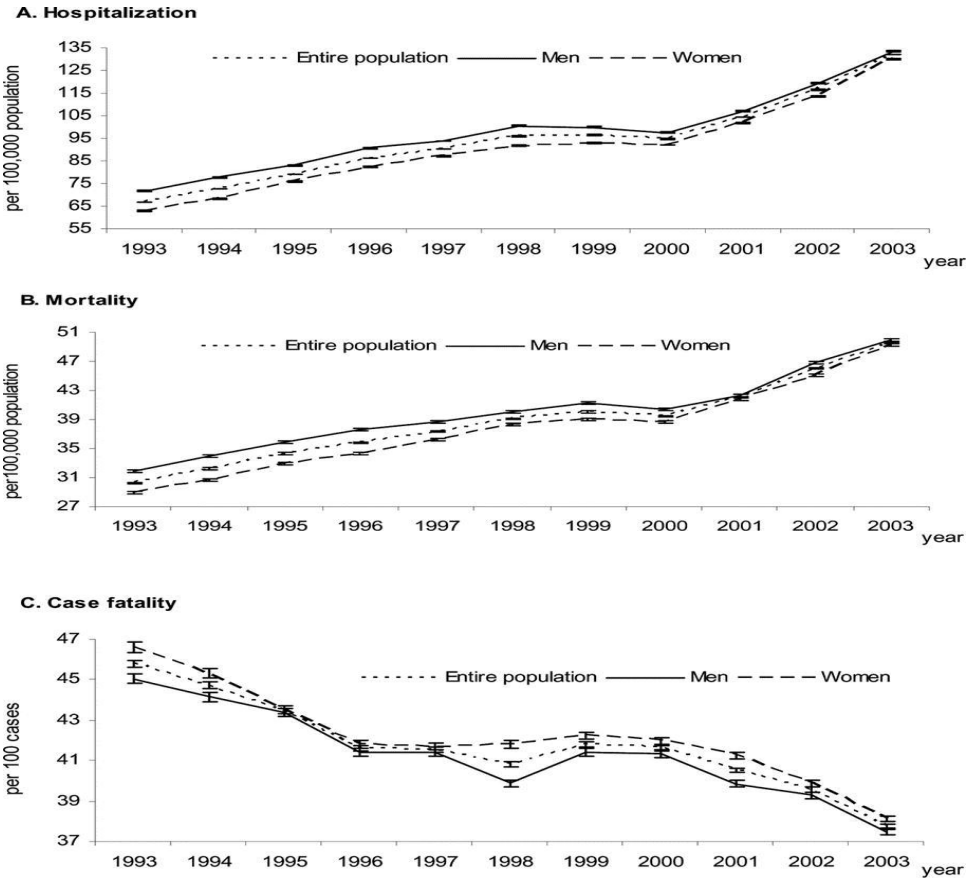



**Figure 3.** Age-adjusted hospitalization (A), mortality (B) and case fatality (C) rates for severe sepsis in the United States in 1993–2003. Adopted from Dombrovskiy et al. 2007 b with permission.

Age is also an independent predictor of outcome in severe sepsis and mortality increases linearly by age (Angus et al. 2001 a; Martin et al. 2006). Mortality in male gender may be lower (Alberti et al. 2003; Dombrovskiy et al. 2007 b) or higher compared to females (29.3% vs. 27.9%), but differences in underlying disease and site of infection may explain this finding (Angus et al. 2001 a). However, gender has had no influence on mortality in some studies (Wichmann et al. 2000; Engel et al. 2007).

The effect of race on mortality in severe sepsis has been investigated in large retrospective studies in USA. The study by Martin found the highest sepsis mortality in black men (Martin et al. 2003). A recent large study on racial variation between white, black and Hispanic patients found that the highest overall mortality and case fatality was among

black patients and the difference was obvious already among young adults. The mortality in Hispanic patients did not differ from the mortality in white patients in spite of lower incidence (Barnato et al. 2008). Black patients had the highest age-and gender-adjusted hospital case fatality compared with Hispanics or whites (26.1%, 24.6% and 24.2% respectively). Black patients were less likely to receive intensive care, but also had the highest mortality when treated in intensive care (32.1% for blacks, 29.3% for whites, p<0001). Black patients were treated in hospitals with lower hospital performance in severe sepsis compared to white patients and this might explain the higher case fatality according to the investigators. However, one earlier study reported similar case fatality between black patients and white patients and no disparities in the quality of treatment, but population-based mortality was higher in blacks in this study, too (Dombrovskiy et al. 2007 a).

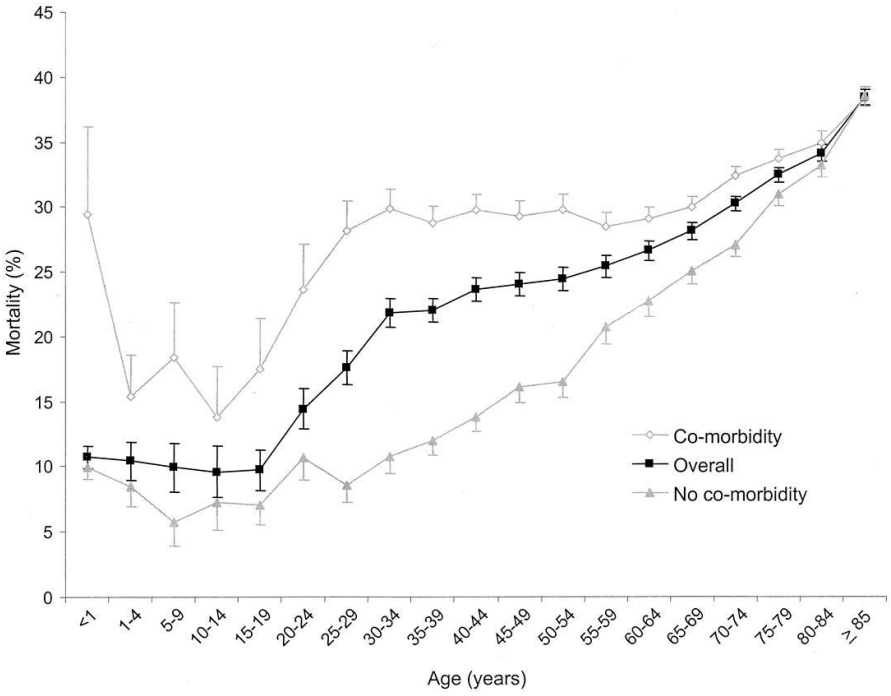



**Figure 4**. Age-specific mortality rates for all cases of severe sepsis and for those with and without underlying comorbidity in the United States. Adopted from Angus et al. 2001 a with permission.

Not surprisingly, co-morbidities such as chronic liver failure, chronic heart failure and immunosuppression in patients with severe sepsis have an exacerbating effect on mortality (Angus et al. 2001 a; Alberti et al. 2003; Esper et al. 2006). The influence of age and co-morbidities on mortality is shown in Figure 4.

The number of dysfunctioning or failing organs has a strong influence on mortality. The ICU mortality with four organ failures was ten-fold compared to patients with one organ failure in the European Sepsis Study (Vincent et al. 2006). The hospital mortality in patients with at least two organ failures is four-hold compared to patients with only one organ failure (49% vs. 11% respectively) (Guidet et al. 2005). Especially hepatic, renal and respiratory failures are associated with poor outcome (Angus et al. 2001 a).

Except gender and race, other genetic factors such as gene polymorphism have also been shown to have influence on mortality in severe sepsis. Different genotypes such as IL-1α and IL-1β genes (Ma et al. 2002), IL-1 receptor antagonist gene (Arnalich et al. 2002; Ma et al. 2002), CD14 promoter gene (Gibot et al. 2002), mannose-binding lectin (Gordon et al. 2006) and protein C-1641 (Walley et al. 2007) have been found to affect mortality in human studies.

The performance of individual intensive care departments on treating patients with severe sepsis has an impact on mortality. This variation in outcome has been found between countries (Vincent et al. 2006), private and community hospitals (Silva et al. 2004) or analogous hospitals within the same country (Yu et al. 2003). The differences in outcome can be accounted for by the disparities between patients and severity of illness, but also by different treatment modalities (Yu et al. 2003). Finally, decisions to withhold or withdraw intensive care and life-sustaining treatments are most often taken on patients with severe co-morbidities or incurable acute illness. These decisions have significant influence on short-term outcome in patients with critical illness (Azoulay et al. 2003).

### 2.5.2 Long-term mortality

*"The most important endpoint by which to evaluate the outcome of critically ill patients is long term survival" (Williams et al. 2008)*

Patients surviving critical care are involved with increased risk of death for the next 15 years compared with the general population (Williams et al. 2008). Age, co-morbidities and primary diagnosis have the greatest effect on the long-term outcome in patients with critical illness in general (Niskanen et al. 1996; Williams et al. 2008) and have been shown to have influence on patients with severe sepsis as well (Weycker et al. 2003).

Until now most studies on severe sepsis have been restricted to short-term outcome such as ICU and hospital mortality. However, long-term outcome evaluation should be included in clinical trials in addition to short-term follow-up (Angus et al. 2003 a). One-year mortality has been as high as 51.4% and five-year mortality 74.2% among patients with severe sepsis when the majority of patients have been over 65 years old (Weycker et al. 2003). Even with younger patients, two-year mortality is up to 57% in patients with severe sepsis (Korošec Jagodič et al. 2006). Patients with severe sepsis have decreased long-term survival compared to patients with other critical illness (Granja et al. 2004) or with trauma (Korošec Jagodič et al. 2006).

### 2.5.3 Quality of life, quality-adjusted life years and cost of treatment

*Quality of life*

The outcome after critical illness comprises not only survival but also quality of life. It is important to evaluate quality of life (QOL) when assessing long-term outcome in patients with severe sepsis (Marshall et al. 2005). Critical illness and its sequelae have an influence on QOL. Prolonged neuropsychological impairment has been found in survivors of intensive care (Jackson et al. 2003; Jones et al. 2006) and experimental animal studies have shown learning and memory impairment after sepsis (Barichello et al. 2008; Tuon et al. 2008).

There is no consensus on the time most suitable for QOL assessment (Black et al. 2001), but a minimum of 6 months after critical illness has been recommended (Angus et al. 2003 a). QOL has been evaluated within 6-12 months after hospital discharge (Niskanen et al. 1999; Eddleston et al. 2000; Graf et al. 2005) and even in 5-12 years after treatment in intensive care (Flaatten et al. 2001; Graf et al. 2005; Kaarlola et al. 2006). These studies with general ICU patients have also included patients with severe sepsis. Studies limited to patients with severe sepsis have assessed QOL at 6 months (Granja et al. 2004), 16 months (Heyland et al. 2000) or 2 years (Korošec Jagodič et al. 2006).

Various instruments have been used to measure QOL in patients with critical illness. The Nottingham Health Profile (NHP) (Hunt et al. 1980), EuroQol (EQ-5D) (The EuroQol Group. 1990), the Medical Outcome Study 36-Item Short Form Health Survey (MOS SF-

36) (Ware et al. 1992) and the RAND 36-Item Health Survey 1.0 (RAND-36) (Hays et al. 1993) have been most widely used in studies concerning heath-related QOL (HR-QOL) in patients with critical illness in general (Niskanen et al. 1999; Eddleston et al. 2000; Flaatten and Kvåle 2001; García Lizana et al. 2003; Cuthbertson et al. 2004; Graf et al. 2005; Kaarlola et al. 2006; Hofhuis et al. 2007) and specifically with severe sepsis (Heyland et al. 2000; Weycker et al. 2003; Granja et al. 2004; Korošec Jagodič et al. 2006).

NHP, MOS SF-36 and RAND-36 are generic health profile measures. Health profiles provide various outcome scores on different QOL domains. They allow assessment of different conditions or treatments on the whole profile or one specific QOL domain. NHP comprises 6 domains: energy level, emotional reactions, physical mobility, pain, social isolation and sleep (Hunt et al. 1980). MOS SF-36 comprises 8 domains: physical functioning, bodily pain, role limitations due to physical problems, role limitations due to emotional problems, general mental health, social functioning, energy/fatigue, and general health perceptions (Ware and Sherbourne 1992). RAND-36 actually includes the same domains as those in the SF-36, but the scoring algorithm is different from that of the SF-36 (Hays et al. 1993).

Generic preference-based measures express the subjective health status with one health index score. The index score is a single number, which is usually a continuum from perfect health to worst outcome (1-0). The index score can be used in cost-utility analyses to calculate quality-adjusted life-years (QALY) (Torrance et al. 1989). EQ-5D is a generic preference-based instrument, which comprises 5 domains: mobility, self care, usual activities, anxiety and/or depression and bodily pain (The EuroQol Group 1990). In addition, it also includes a visual analogue scale (VAS) on the current health state.

MOS SF-36 and EQ-5D have been evaluated for reliability and validity in various studies (Brazier et al. 1993; van Agt 1994; Chrispin et al. 1997). None of above mentioned four instruments has been found superior to the others (Essink-Bot et al. 1997). However, in critical care setting, SF-36 and EQ-5D are recommended by the 2002 Brussels Roundtable (Angus et al. 2003). SF-36 serves as a comprehensive health profile measure and EQ-5D with its single number health index can be used in QOL assessment and in cost-utility analyses. Relatives or next of kin are able to answer reliably on QOL measures on behalf of the patients (Rogers et al. 1997; Hofhuis et al. 2003).

QOL before critical illness has been evaluated in some studies, and poor QOL has been correlated with poorer outcome (Cuthbertson et al. 2005). Quality of life before intensive care admission may even be equal to APACHE II score in predicting outcome (Hofhuis et al. 2007). Severe sepsis is associated with organ failures, from which surviving patients may recover slowly or have some residual dysfunction. Patients with ARDS (Angus et al. 2001 b) and severe acute respiratory syndrome (SARS) (Tansey et al. 2007) have exhibited reduced QOL after one year of critical illness. The ARDS Study excluded septic patients. Acute renal failure has a clear impact on deteriorating QOL and reducing long term outcomes (Åhlström et al. 2005). While the QOL measured by EQ-5D is reduced after major trauma and severe sepsis, there are no differences between the groups after two years (Korošec Jagodič et al. 2006).

*Quality-adjusted life year and cost of treatment*

A quality-adjusted life year (QALY) comprises length of life and quality of life. The concept of QALY enables comparisons of the efficacies of different treatments and calculations of costs per one QALY (Torrance et al. 1989; Kerridge et al. 1995). Cost-effectiveness analysis measures the benefits of treatments in terms of the number of years of lives saved. Cost-utility analysis measures treatments using a number of QALYs as a unit of efficacy (Anonymous 2002). In intensive care medicine, the generally accepted cost for one life year gained or for one QALY has been $50,000 (Talmor et al. 2006). Examples of the cost per one QALY in different treatments are presented in Table 8.

**Table 8.** Cost per one QALY in different treatments.

| Reference | Treatment | Cost per 1 QALY |
|---|---|---|
| Añón et al. 1999 | Respiratory failure in patients with COPD and oxygen therapy and mechanical ventilation | $26 300-$44 600 |
| Mayer et al. 2000 | Patients hospitalized for stroke with mechanical ventilation | $174 200 |
| Hamel et al. 2000 | Acute respiratory failure (pneumonia or ARDS) with mechanical ventilation | $29 000-$110 000 |
| Sznajder et al. 2001 | Intensive care treatment in general | $4 100 |
| CDC Diabetes Cost-effectiveness Group 2002 | Reduction of type II complications<br>-with intensive glycemic control<br>-with intensified hypertension control<br>-with reduction in serum cholesterol level with pravastatin | <br>$41 400<br>$2000<br>$51 900 |
| Manns et al. 2002 | Severe sepsis with activated protein C<br>-all patients<br>-APACHE II >25<br>-APACHE II <25 | <br>$46 600<br>$32 900<br>$958 400 |
| Angus et al. 2003 b | Severe sepsis with activated protein C | $48 800 |
| Fowler et al. 2003 | Severe sepsis with activated protein C<br>APACHE II >25<br>APACHE II <25 | <br>$13 500<br>$403 000 |
| Graf et al. 2005 | Intensive care treatment in medical ICU | $21 900 |
| Green et al. 2006 | Severe sepsis with activated protein C | £8200 |
| Ridley et al. 2007 | Intensive care treatment in general | £7000 |
| Dhainaut et al. 2007 | Severe sepsis with activated protein C | 33 800 € |
| Graf et al. 2008 | Cardiac arrest with cardiopulmonary resuscitation | 11 600 € |
| Talmor et al. 2008 | Integrated sepsis protocol for severe sepsis | $16 300 |

# 3 AIMS OF THE STUDY

The objective of this study was to evaluate the incidence, clinical course and short- and long-term outcomes of severe sepsis in adult patients treated in intensive care. The specific aims were

1. To determine the incidence, associated organ dysfunctions and outcome of severe sepsis in the adult Finnish population and to evaluate the compliance with treatment guidelines in clinical practice (Study I).

2. To study the predictive value of high mobility group box-1 protein (HMGB1) and hospital mortality in adult patients with severe sepsis and to evaluate the relation of HMGB1 to severity of illness and development and type of organ dysfunction (Study II).

3. To evaluate the relationship between vascular endothelial growth factor (VEGF) and organ dysfunction and the value of VEGF in the prediction of hospital mortality (Study III).

4. To determine long-term survival and quality of life (QOL) after severe sepsis using a generic EuroQol (EQ-5D) questionnaire and to calculate quality-adjusted life-years (QALYs) and costs per QALY for this patient group (Study IV).

# 4 PATIENTS AND METHODS

## 4.1. PATIENTS

Four-hundred seventy patients were included in the Finnsepsis Study in 24 different ICUs in 21 hospitals within a 4-month period. Consent was waived for collecting the severe sepsis data but consent for the use of laboratory samples (for Studies II and III) was needed within the first 24 hours and for quality of life (QOL) assessment (for Study IV) in the first week after study entry. All patients participated in Study I and 250/470 patients participated in Studies II-III. Samples for Study II were not available for analysis in 3 patients and the final number of patients in study II was 247. In Study IV, 252/470 patients with consents participated in QOL assessment before severe sepsis and 156 patients participated in QOL assessment after severe sepsis. The flowchart of included patients in Studies I-IV is presented in Figure 5.

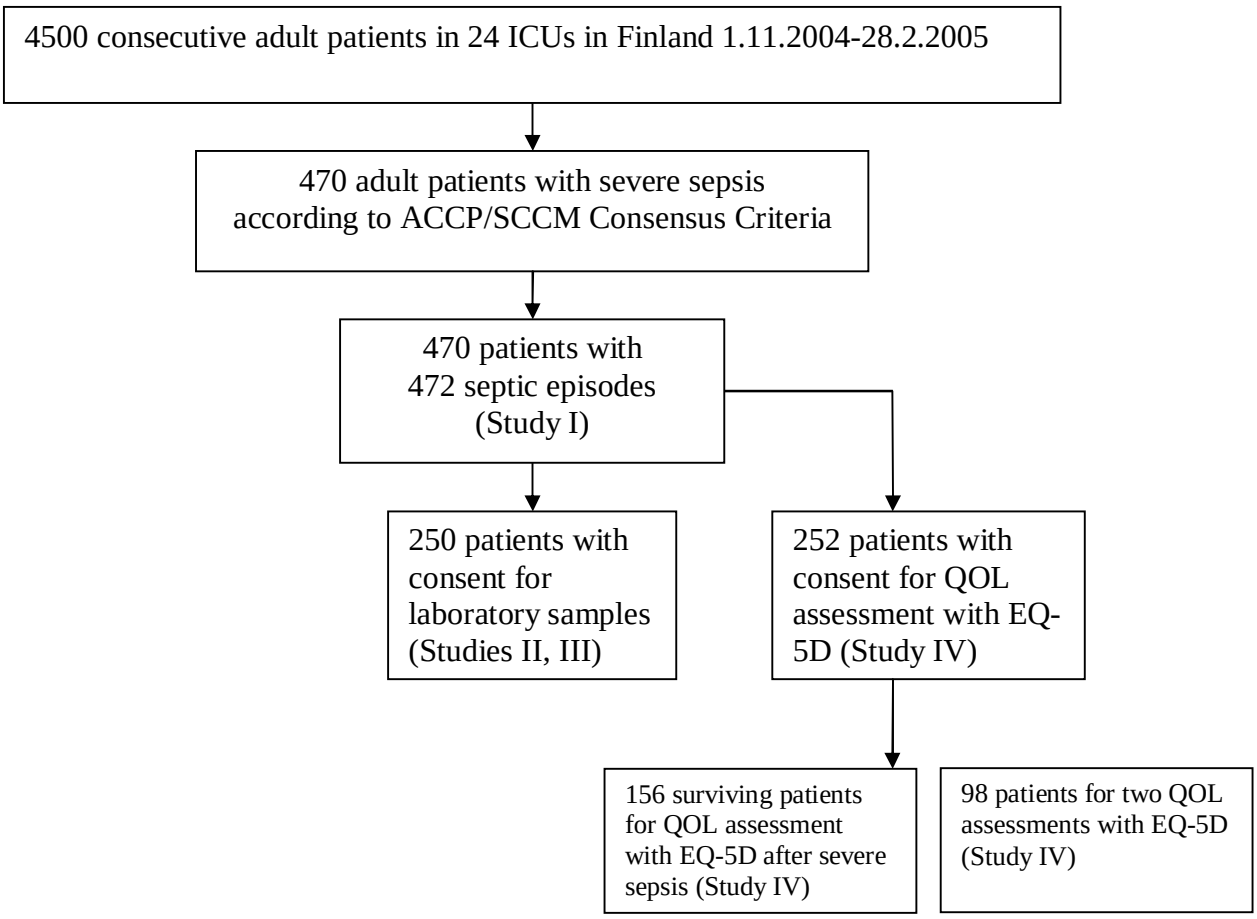



**Figure 5.** The flowchart of inclusion in studies I-IV.

In addition, 30 healthy individuals in Study II and 10 in Study III served as a control group. Healthy individuals were hospital employees. The demographic data of the patients in Studies I-IV are presented in Table 9.

**Table 9**. The demographic data of study patients.

| | **Study I** | **Study II** | **Study III** | **Study IV** |
|---|---|---|---|---|
| Patient N | 470 | 247 | 250 | 156 |
| Male N (%) | 315 (67) | 171 (69.2) | 172 (68.8) | 114 (73.1) |
| Age, mean (SD) | 59.6 (15.2) | 59.3 (15.6) | 59.4 (15.6) | 58.6 (15.8) |
| APACHE II mean (SD) | 24.1 (9.1) | 23.8 (9.1) | 23.8 (9.1) | 22.3 (7.7) |
| SAPS II mean (SD) | 44.8 (16.9) | 43.5 (16.9) | 43.5 (16.8) | 41.1 (14.3) |
| SOFA at 24h, mean (SD) | 8.4 (3.6) | 10.9 (4.3) | 10.9 (4.3) | 8.2 (3.2) |
| Post-operative N (%) | 136 (29) | 65 (26.3) | 65 (26.0) | 41 (26.3) |
| ICU mortality N (%) | 73 (15.5) | 33(13.4) | 33(13.2 | |
| Hospital mortality N (%) | 133 (28.3) | 65 (26.3) | 66 (26.4) | |

## 4.2 METHODS

### Study designs

**Study I.** Study I was a prospective observational cohort study. The purpose of the study was to determine the incidence, associated organ failures and outcome on severe sepsis in adult Finnish population. We also were interested to evaluate how some recommended treatment guidelines in severe sepsis were applied. All consecutive ICU admission episodes (4500) were screened for severe sepsis in a 4-month period (from 1 November 2004 to 28 February 2005). Severe sepsis was diagnosed according to ACCP/SCCM Consensus Criteria (Bone et al. 1992). These criteria were fulfilled in 470 patients and all these patients were included in the first study. Study entry was the time when all three criteria were met. If the patient had more than one severe sepsis episode from the same source, only the first period was taken for severe sepsis incidence, treatment and outcome analysis, but organ dysfunctions were assessed for all ICU days. If the patient had more than one severe sepsis episode from different sources, all periods were included in all analyses except mortality. Eleven hospitals screened patients with antibiotic treatment for severe sepsis on the wards on 4 separate predetermined screening days. Demographic data and severity of illness scoring systems such as Simplified Acute Physiology Score (SAPS) II score (Le Gall et al. 1993) and Acute Physiology and Chronic Health Evaluation

(APACHE) II score (Knaus et al. 1985) were recorded. Haemodynamic data were collected in the first 24 hours. Data concerning blood cultures, source of infection and use of antimicrobial treatment were collected and the severity of organ dysfunction was assessed daily by Sequential Organ Failure Assessment (SOFA) score (Vincent et al. 1998). Adjuvant treatments, e.g. vasoactive and ventilator therapies and renal replacement therapies were recorded, as well as the use of rhAPC and low-dose hydrocortisone. Length of stay (LOS) in ICU and hospital and ICU and hospital mortalities were assessed. One-year mortality data was obtained from Statistics Finland.

**Study II.** Study II was a substudy of the main study, where high mobility group box 1 protein (HMGB1) was analysed. HMGB1 was studied as a biomarker for severe sepsis and severity of organ failure and its usefulness in outcome was tested. Serum samples were analysed by semi-quantitative Western immunoblotting (WB) analysis in 247 patients in the first 24 hours and in 210 patients 72 hours later. The results were confirmed by enzyme-linked immunosorbent assay (ELISA) in 170 randomly selected patients. Blood samples from 10 healthy volunteers were analysed as controls with both methods.

**Study III.** Study III was a substudy of the main study, where vascular endothelial growth factor (VEGF) was tested as a biomarker for severity of illness, organ dysfunction and outcome. Serum samples were analysed by ELISA in 250 patients in the first 24 hours and 215 patients after 72 hours. Blood samples from 30 healthy volunteers were analysed as controls.

**Study IV.** In the fourth study the purpose was to ascertain two-year survival and health-related quality of life (HR-QOL) in Finnsepsis Study patients. A generic EuroQol (EQ-5D) (The EuroQol Group 1990) instrument was used for QOL assessment. QOL before critical illness was assessed in 252 patients (54%) and the measurement was repeated in a median of 17 months after severe sepsis. One-hundred and fifty-six patients (58%) responded the second measure and of those 98 patients (63%) had also participated in the first measure. Quality-adjusted life-years (QALY) and cost per QALY were also estimated for non-respondents. The QOL data of the respondents were assumed to be comparable for the non-respondents, and thus the mean EQ-5D data of age- and gender-matched respondents were used for QALY assessment in non-respondents. The mean cost for one QALY was

calculated by using the mean ICU and the hospital cost of the study patients and dividing the sum by acquired QALYs gained.

### 4.3 SCORING METHODS FOR THE SEVERITY OF ILLNESS

Severity of acute illness was assessed using the Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al. 1985) and Simplified Acute Physiology Score (SAPS) II (Le Gall et al. 1993), which were calculated in the first 24 hours at ICU admission. Organ dysfunction or failure was assessed daily using Sequential Organ Failure Assessment (SOFA) scores (Vincent et al. 1998). SOFA scores were calculated in the first 24 hours at ICU admission (SOFA at 24 h) and thereafter daily after study inclusion using the poorest values during previous 24 hours. Missing organ specific SOFA score was not replaced and the score was assumed to be zero. Maximum SOFA scores (SOFAmax) (Moreno et al. 1999) were used to indicate the worst grade of sepsis-related organ dysfunction. SOFA score 1-2 was graded as organ dysfunction and SOFA score 3-4 as organ failure.

### 4.4 LABORATORY ASSAYS

**Blood samples (II and III)**. Arterial blood samples for HMGB1 and VEGF analyses were drawn after informed consent within 24 hours of the study entry (day 0) and 72 hours thereafter. Blood for serum samples was collected in glass vacuum tubes (BD Vacutainer 369032) and the samples were prepared within 60 minutes of sampling. More specifically, after 30-45 minutes of serum separation at room temperature the samples were centrifuged at 3100 rpm (~1500 G) for 15 minutes. The samples were stored at –70ºC and transferred from local hospitals to Helsinki University Hospital for storage and subsequent analyses.

**HMGB1 analysis (II).** In the Study II, HMGB1 concentrations were first measured by Western immunoblotting (WB). Control samples were drawn from 10 healthy volunteers. Because we failed to obtain a pure HMGB1 control, a pooled serum sample was used as an inter-assay (inter-gel) control for the semi-quantification of HMGB1 levels in serum. The HMGB1 signal on each gel was 'normalized' against the pooled serum sample from healthy volunteers. The HMGB1 signal from the individual healthy volunteers (controls) was similarly normalised against the pooled samples. Arbitrary units of HMGB1 activity (%) on the gels were reported and used for statistical analyses. After a commercial enzyme-

linked immunosorbent assay (HMGB1 ELISA Kit II; Shino-Test Corporation, Kanagawa, Japan) became available, the blood samples of 10 healthy controls and day 0 samples of a randomly generated subgroup of patients (N=170) were re-analysed by ELISA. Analyses were measured in the University of Kuopio.

**VEGF analysis (III).** In the third study, VEGF was analysed by ELISA. Control samples were drawn from 30 healthy volunteers. VEGF concentrations in sera were measured in duplicate for each sample using a commercial enzyme-linked immunosorbent assay kit (Quantikine®, R&D Systems; Minneapolis, MN) that recognizes the soluble isoforms ($VEGF_{121}$ and $VEGF_{165}$). Analyses were measured in the University of Kuopio.

## 4.5 INTERVENTIONS

Patients were treated according to general accepted guidelines and protocols without special therapeutic interventions except blood sampling and QOL assessment (EQ-5D) after consent.

## 4.6 DATA COLLECTION

Data were collected daily at the participating hospitals by local study nurses and investigators. All data were transferred and stored via the Internet in the Finnish Intensive Care Quality Consortium (Intensium Ltd., Kuopio) database.

*Data management.* All data were collected to the internet based database. Every sixth patient was rechecked for inclusion criteria at study entry and survival status at hospital discharge. In this recheck, each patient fulfilled the inclusion criteria. One ICU survivor was registered as non-survivor, and this was corrected. One year mortality data was obtained from Statistics Finland.

## 4.7 OUTCOME MEASURES

### Mortality (I, II, III, IV)

*Short term mortality* (I, II, III) comprised ICU and hospital mortality. The data were obtained from the hospital records in the participating hospitals.

*Long term mortality* (I, IV) comprised one-year mortality and two-year mortality. The data was obtained from Statistics Finland in May 2006 and in May 2007.

### Quality of life (QOL) (IV)

Quality of life was assessed using generic, utility and preference-based EuroQol (EQ-5D) instrument (The EuroQol group 1990). The EQ-5D measure consists of five different domains: mobility (MO), self-care (SC), usual activities (UA), pain/disorder (PD), and anxiety/depression (AD). Respondents were asked to choose the best suitable option from three alternatives [no (=1), moderate (=2), or severe (=3) problems]. Five chosen options created a serial number, from which one summary index (EQ sum index) was calculated. All dimensions had the same weight only in the best category, worse categories had different values depending on dimension. We used general coefficients and reference values in Finnish population (Ohinmaa and Sintonen 1996). In addition, the EQ-5D instrument also comprised a 20-cm visual analogue scale, EQ VAS. Respondents are asked to indicate their present health state by this scale, ranging from “0” (worst imaginable health state) to “100” (best imaginable health state). EQ-5D domains, options and VAS are presented in Table 10.

**Table 10.** EQ-5D questionnaire.

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today

**Mobility**

| | |
|---|---|
| I have no problems in walking about | r |
| I have some problems in walking about | r |
| I am confined to bed | r |

**Self-Care**

| | |
|---|---|
| I have no problems with self-care | r |
| I have some problems washing and dressing myself | r |
| I am unable to wash or dress myself | r |

**Usual activities** (e.g. work, study, housework, family or leisure activities)

| | |
|---|---|
| I have no problems with performing my usual activities | r |
| I have some problems with performing my usual activities | r |
| I am unable to perform my usual activities | r |

**Pain/Discomfort**

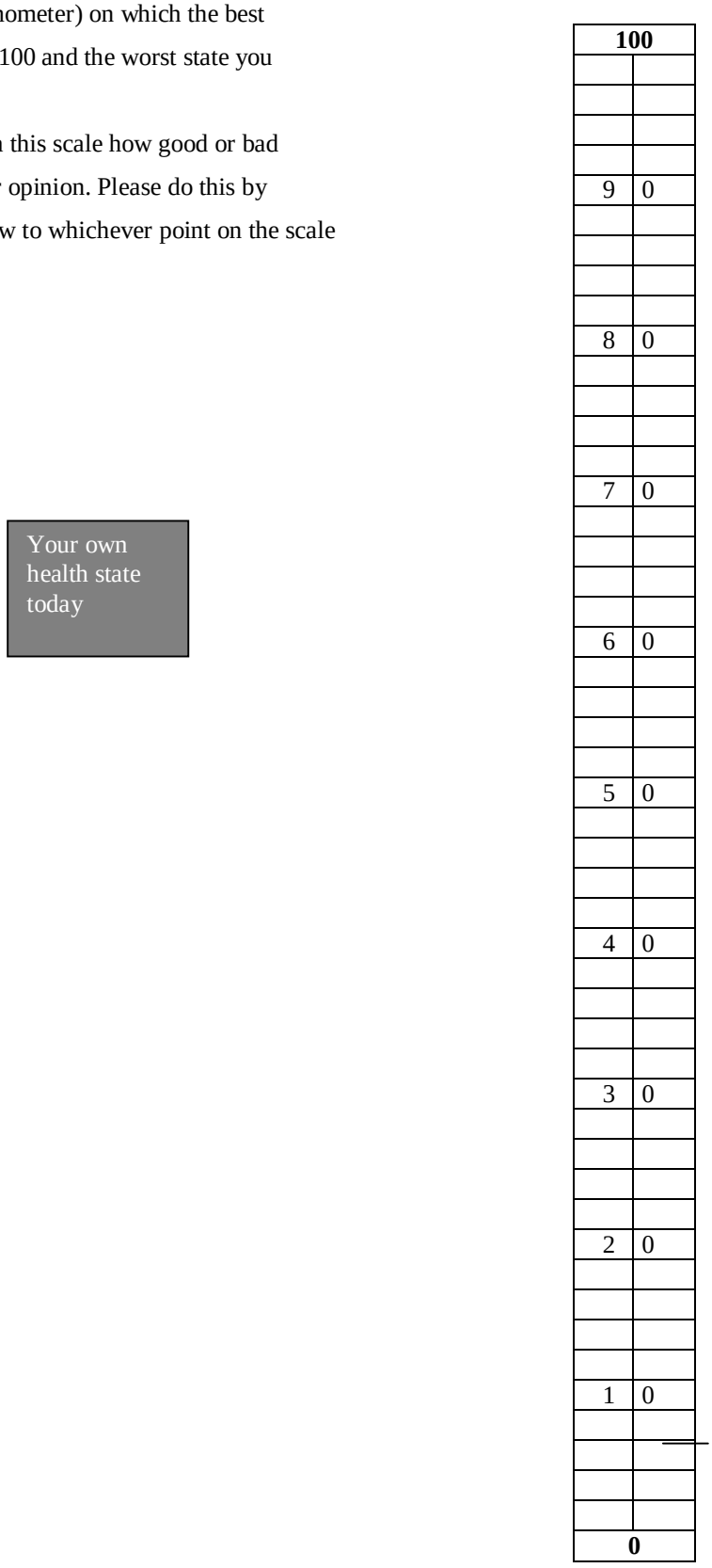
| | |
|---|---|
| I have no pain or discomfort | r |
| I have moderate pain or discomfort | r |
| I have extreme pain or discomfort | r |

**Anxiety/Depression**

| | |
|---|---|
| I am not anxious or depressed | r |
| I am moderately anxious or depressed | r |
| I am extremely anxious or depressed | r |

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale



**Quality-adjusted life year (QALY) (IV)**

A quality-adjusted life year (QALY) comprises the length of life (the number of years of lives saved) and quality of life. Patients' QALY units were calculated at the end of the first quarter of the year 2007 by multiplying the time (years) the patient survived after hospital discharge by the value of the patient's EQ sum index. QALY units for the patients still alive at that time point were calculated using the expected life years of the age- and gender-adjusted Finnish population in the year 2005. QALY units were also estimated for non-respondents and thus for all Finnsepsis patients. The mean EQ5D data of age- and gender-matched respondents were used for estimating QALY of non-responders. This was based on the assumption that the QOL data of the respondents was comparable to the data of the non-responders.

If the patient died during the same hospital stay, QALY was defined as zero. Two examples of QALY calculation are shown: The first patient lived 0.9 years after hospital discharge and her measured EQ sum was 0.63 (EQsum index/100). The calculated QALY will be 0.9 x 0.63 = 0.57. A second patient (male, 41 years) was alive in March 2007. His EQ-sum was 0.69 (EQsum index/100) and his age- and gender-adjusted life expectancy of general population is 36.3 years. QALY = 36.3 x 0.69 = 25.0.

## 4.8 STATISTICAL ANALYSES

**Data** are presented as absolute numbers (percentages), means (standard deviation, SD or confidence interval, CI) or medians and interquartile range (25th to 75th percentiles, interquartile range, IQR).

**Statistical significance**. A level of $p<0.05$ was considered statistically significant in all tests in studies I, II and III. The level of $p<0.01$ was considered statistically significant in all tests in the study IV due to multiple comparisons. All statistical analyses were performed using SPSS 12.1 (I), SPSS 14.0 (III) or SPSS 15.0 (II, IV) software (SPSS Inc, Chicago, Illinois).

**Chi-Square test** was used for categorical variables in all studies (I-IV).

**Kaplan-Meier survival analysis** was used to investigate one-year (I) and two-year (IV) survival in different age groups in Studies I and IV.

**Kruskall-Wallis test** was used to compare continuously distributed data in more than two independent populations. It was used to test the demographic and other data between patients with first or second responses or with non–respondents in Study IV.

**Mann-Whitney U-test** was used compare continuously distributed data between two independent populations. It was used to test patients´ demographic data (I-IV) and the laboratory data between survivors and non-survivors in Studies II and III.

**Receiver Operating Characteristic curve (ROC) analysis** was used to determine the prognostic accuracy of HMGB1 and VEGF on both time points. The receiver operating characteristic (ROC) curves were constructed and the areas under the curve (AUC) were calculated with 95% confidence intervals in Studies II and III.

**Spearman's correlation** was used to test the relations between the estimated time of onset of sepsis and changes in HMGB1 (II) and VEGF concentrations (III).

**Wilcoxon's signed matched pair test** was used to compare differences between paired groups. This test was used to analyse EQ-5D sum, EQ VAS, and corresponding reference values in study IV.

### 4.9 ETHICAL ASPECTS

The ethics committees in each hospital approved the study protocol. The need for informed consent was waived from collecting the data for severe sepsis (Study I), but consent was needed for blood samples and quality of life assessments from patients or their next of kin (Studies II, III, IV).

# 5 RESULTS

## 5.1 INCIDENCE OF SEVERE SEPSIS (I)

During the 4-month study period, 4,500 adult patients were treated in 24 intensive care units. Four-hundred and seventy patients suffered 472 episodes of severe sepsis. The severe sepsis was community-acquired in 58% of cases and nosocomial in 39%. Blood cultures were taken from 68% of patients and gram-positive bacteria were found in 59% and gram-negative in 33% of positive blood cultures. Lung and intra-abdominal infections were most common sources of severe sepsis, being responsible for 75% of all cases. The incidence of severe sepsis requiring intensive care was calculated to be 0.38/1,000 adults/year (95% CI 0.34-0.41) in the population, which included 90.6% of Finnish adults. The screening of 4843 adult patients with severe sepsis in the wards gave an additional incidence of 0.31/1,000 adults. Consent for the use of laboratory samples (for Studies II and III) was needed within the first 24 hours and for quality of life (QOL) assessment (for Study IV) in the first week after study entry. Thus the estimated in-hospital incidence of severe sepsis was 0.69/1,000.

## 5.2 ORGAN DYSFUNCTION AND TREATMENTS (I)

Respiratory failure (71.4%) and septic shock (77%) were the most frequent organ dysfunctions. A majority of patients with severe sepsis (87%) needed mechanical ventilation during their treatment period in ICU. Severe oxygenation impairment (SOFA score $\geq$ 3) was found in 71% of the patients. Seventy-seven per cent of patients had a severe cardiovascular failure (SOFA score $\geq$ 3) requiring vasoactive treatment. Acute renal failure (SOFA score $\geq$ 3) was found in 23% of patients. Half of those patients were treated with renal replacement therapies.

Antibiotic treatment was ongoing at hospital admission in 20% of patients with community acquired infection, but the median time for antibiotic treatment after hospital admission was 7 hours (IQR 2.9-15.8) in this patient group. Patients with nosocomial infections were already on antibiotic treatment in 70.5% of cases before severe sepsis was diagnosed. RhAPC was administered only to 15 patients, although we identified at least 40 additional patients with severe sepsis, multiple organ dysfunctions and disseminated intravascular

coagulation (DIC), for whom rhAPC treatment could have been indicated. Low dose corticosteroid treatment was used in less than half of the patients with septic shock, but there was no difference in hospital mortality between these patients. Poorest compliance was found with low tidal volume ventilation (15% in female patients).

### 5.3 IMPACT OF HMGB1 ON ORGAN DYSFUNCTION AND OUTCOME (II)

The mean APACHE II and SAPS II scores in the study patients were 24 (SD9) and 44 (SD17) respectively. The hospital mortality was 26%. Two-hundred and forty seven samples were obtained at baseline (day=0) and 210 samples were taken 72 hours later. All samples were analysed by Western immunoblotting (WB). A subgroup of 170 patients was randomly selected for a reanalysis with ELISA in proportion to survivors (74%) and non-survivors (26%). HMGB1 concentrations analysed by ELISA showed no correlation with concentrations determined by WB (Spearman's rho 0.082, p=0.29). Corresponding HMGB1 values for survivors and non-survivors measured with both methods are shown in Figure 6. This data was not published in Study II.

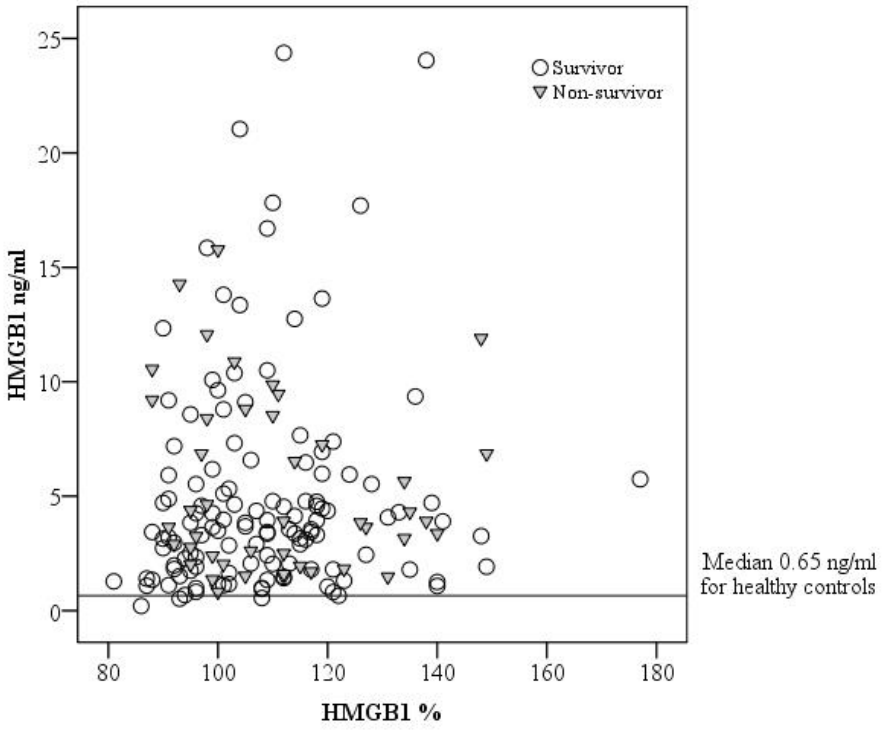



**Figure 6**. HMGB1 levels measured with WB (HMGB1%) and ELISA (HMGB1 ng/ml). The line represents the median concentration for healthy controls analysed by ELISA.

Basic characteristics, disease severity, organ failure and mortality in the HMGB1 study group were similar to other Finnsepsis patients. The median HMGB1 level on day 0 was 108% (IQR 98 and 119) and after 72 hours 107% (IQR 99 and 120). HMGB1 levels were higher than the median HMGB1 level in healthy controls at both time points. The median HMGB1 levels did not differ between survivors and non-survivors on day 0 or 72 hours later. The reanalysis by ELISA confirmed these results. The HMGB1 levels in survivors, non-survivors and healthy controls as measured with both methods are presented in Figure 7.

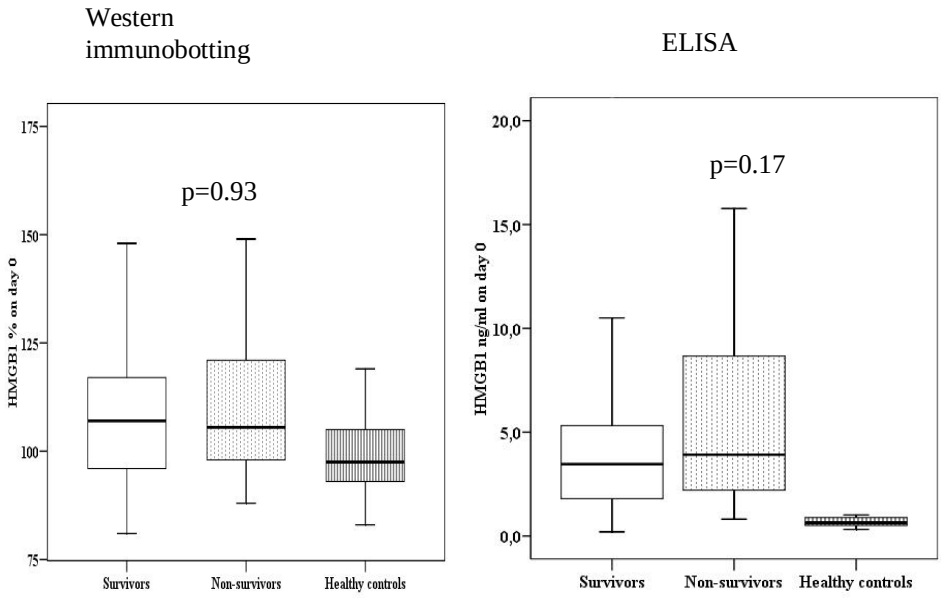



**Figure 7.** HMGB1 levels on day 0 in survivors, non-survivors and healthy controls measured by WB or ELISA (Figures 2A and 2B combined from Study II).

HMGB1 levels and the severity of organ dysfunction (SOFAmax $\leq$ 2) or organ failure (SOFAmax 3-4) did not correlate with the day 0 measurement. At 72 hours, the median HMGB1 levels 103% (IQR 96 and 114) in patients with severe cardiovascular failure (SOFAmax 4) were lower than at study entry (106%, IQR 97 and 117, p=0.001). Patients with severe haematological failure (SOFA 3-4) also had lower HMGB1 concentrations at 72 h than on day 0 (103% [IQR 96 and 115] vs. 109% [IQR 97 and 118] p=0.032]. Levels did not differ in milder dysfunctions or in other organ systems. Low HMGB1 levels were associated with the severity of the total score of organ failure in patients with SOFAmax score 14-24.

HMGB1 levels did not predict hospital mortality. ROC analyses with death as the outcome showed areas under the curve (AUC) 0.51 and 0.56 (95% confidence limits 0.40-0.61 and 0.47-0.65 respectively) (Figure 8). The AUC in the HMGB1 ELISA subgroup was 0.57 (95% confidence limits 0.47 and 0.67) on day 0.

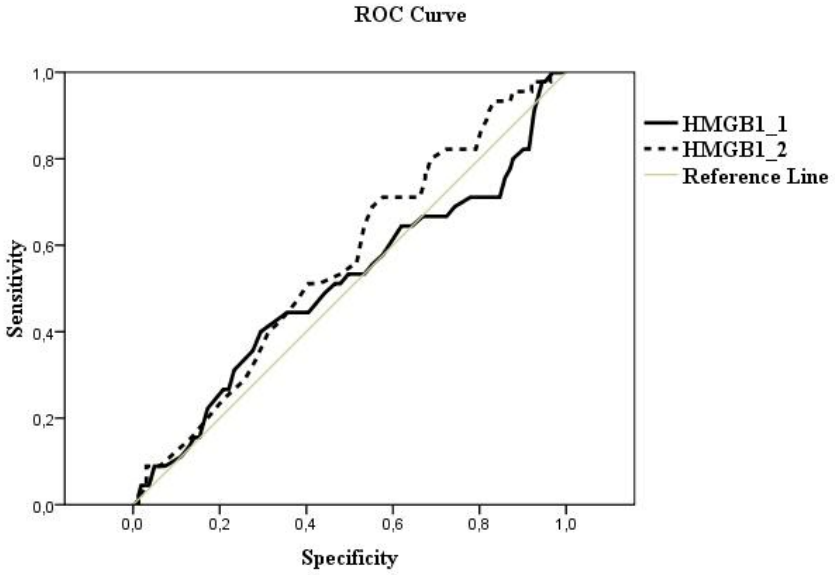



**Figure 8.** ROC curve for HMGB1 in predicting hospital mortality. HMGB1_1 refers to levels on day 0 and HMGB1_2 refers to levels at 72 hours.

## 5.4 IMPACT OF VEGF ON ORGAN DYSFUNCTION AND OUTCOME (III)

The mean APACHE II and SAPS II scores in the study patients were 24 (SD9) and 44 (SD17) respectively. Hospital mortality was 26%. Laboratory samples were obtained from 250 patients at study entry (day 0) and from 215 patients after 72 hours. The median VEGF concentration was 260 pg/ml (IQR 126 and 459 pg/ml) in healthy volunteers. The median VEGF concentration on day 0 (423 pg/ml [IQR 159 and 858 pg/ml]) and after 72 hours (521 pg/ml [IQR 182 and 1092 pg/ml]) were higher in patients with severe sepsis than in healthy volunteers (p=0.03 and p=0.003 respectively). The concentrations were lower in non-survivors at both time points (p=0.012 and p=0.009 respectively). The VEGF concentrations in survivors and non-survivors are presented in Figure 9.

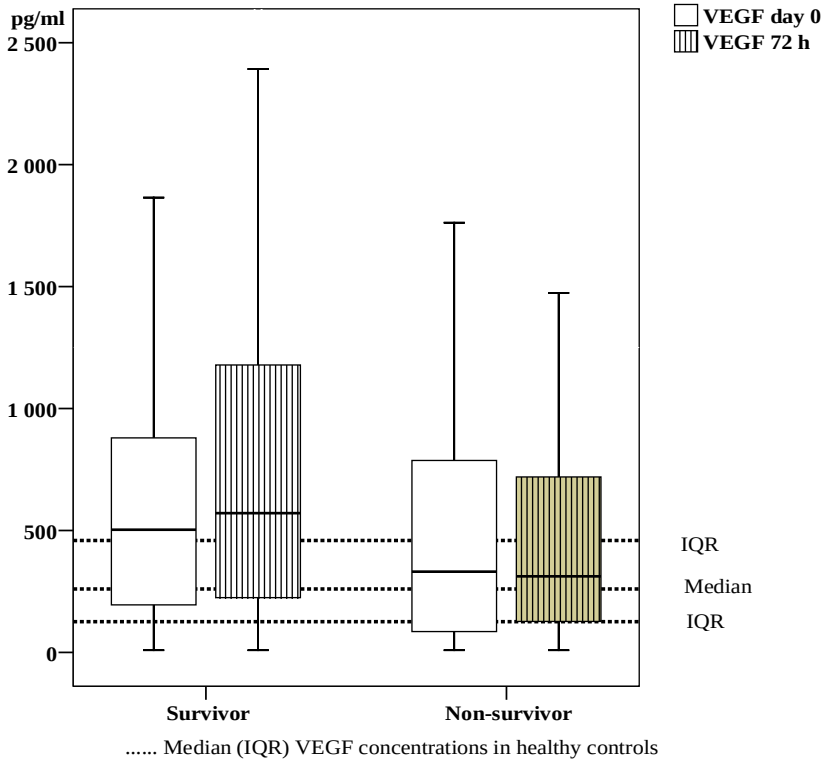



**Figure 9.** VEGF concentrations in survivors and non-survivors. (Figure 1 from Study III).

VEGF concentrations had influence on the severity of organ dysfunction. Patients with severe renal, haematological or liver failure had decreased VEGF concentrations compared to milder dysfunctions (p=<0.002, p=0.00 and p=<0.02 respectively). Cardiovascular or respiratory dysfunctions were not associated with VEGF levels. Accordingly, VEGF concentrations were associated with the severity of overall organ dysfunction. The patients with SOFAmax score 14-24 had lower VEGF levels on both time points (165 pg/ml [IQR 54 and 466] and 208 pg/ml [IQR 52 and 339]) as compared to patients with lower SOFAmax score. In patients with community-acquired severe sepsis, the hospital survivors had increasing VEGF concentrations in contrast to hospital non-survivors with no change or decreasing concentrations.

The overall VEGF concentrations did not predict hospital mortality. The ROC curves (Figure 10) for VEGF levels showed no significant areas under the curve (AUC). The AUC for day 0 VEGF was 0.58 (95% confidence limits 0.48 and 0.68) and for VEGF at 72 hours 0.63 (95% confidence limits 0.54 and 0.72).

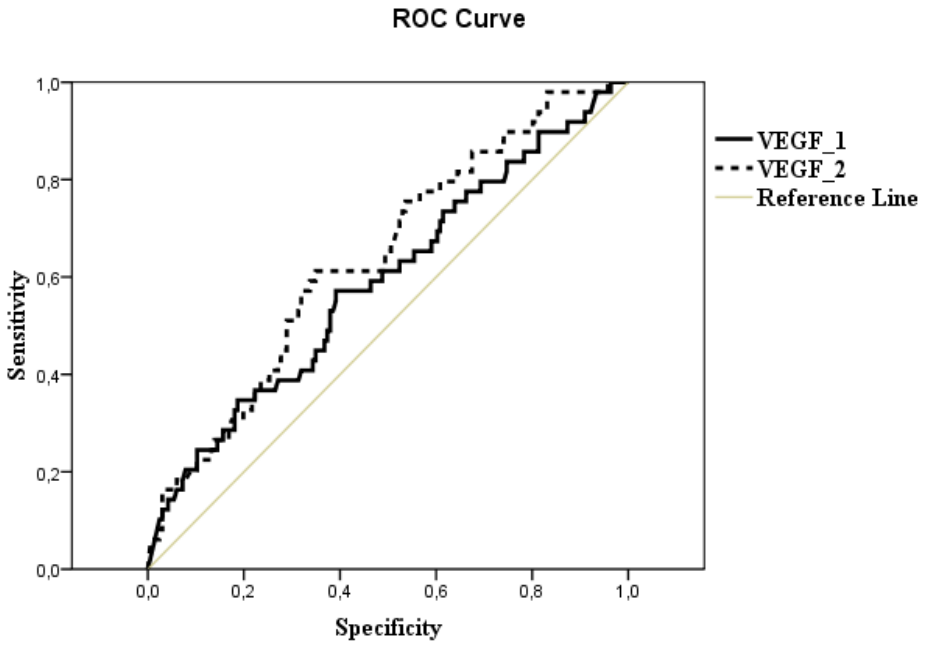



**Figure 10.** ROC curve for VEGF in predicting hospital mortality. VEGF_1 refers to concentrations on day 0 and VEGF_2 refers to concentrations at 72 hours.

## 5.5 CORRELATION BETWEEN HMGB1, VEGF AND ORGAN DYSFUNCTION

The concentrations between HMGB1 and VEGF on day 0 and after 72 hours correlated poorly when HMGB1 was measured with WB (Spearman's rho 0.26 and 0.36 respectively). Nor was any correlation found between HMGB1 analysed by ELISA and VEGF on the first day (Spearman's rho 0.055). Some survivors had high HMGB1 levels and only moderately high VEGF concentrations, as shown in Figure 11.

Low VEGF concentrations associated with haematological failure (SOFA score 3-4) had only moderate correlation with HMGB1 levels measured by WB (Spearman's rho 0.375). In 40 patients with severe haematological failure, the HMGB1 concentration measured by ELISA showed no correlation with VEGF concentrations (Spearman's rho -0.073). Forty-nine patients with acute renal failure (SOFA score 3-4) and low VEGF concentrations had only moderate correlation with HMGB1 levels on day 0 and at 72 hours (Spearman's rho 0.375 and 0.47 respectively), but no correlation with ELISA-measured HMGB1 (Spearman's rho 0.091).

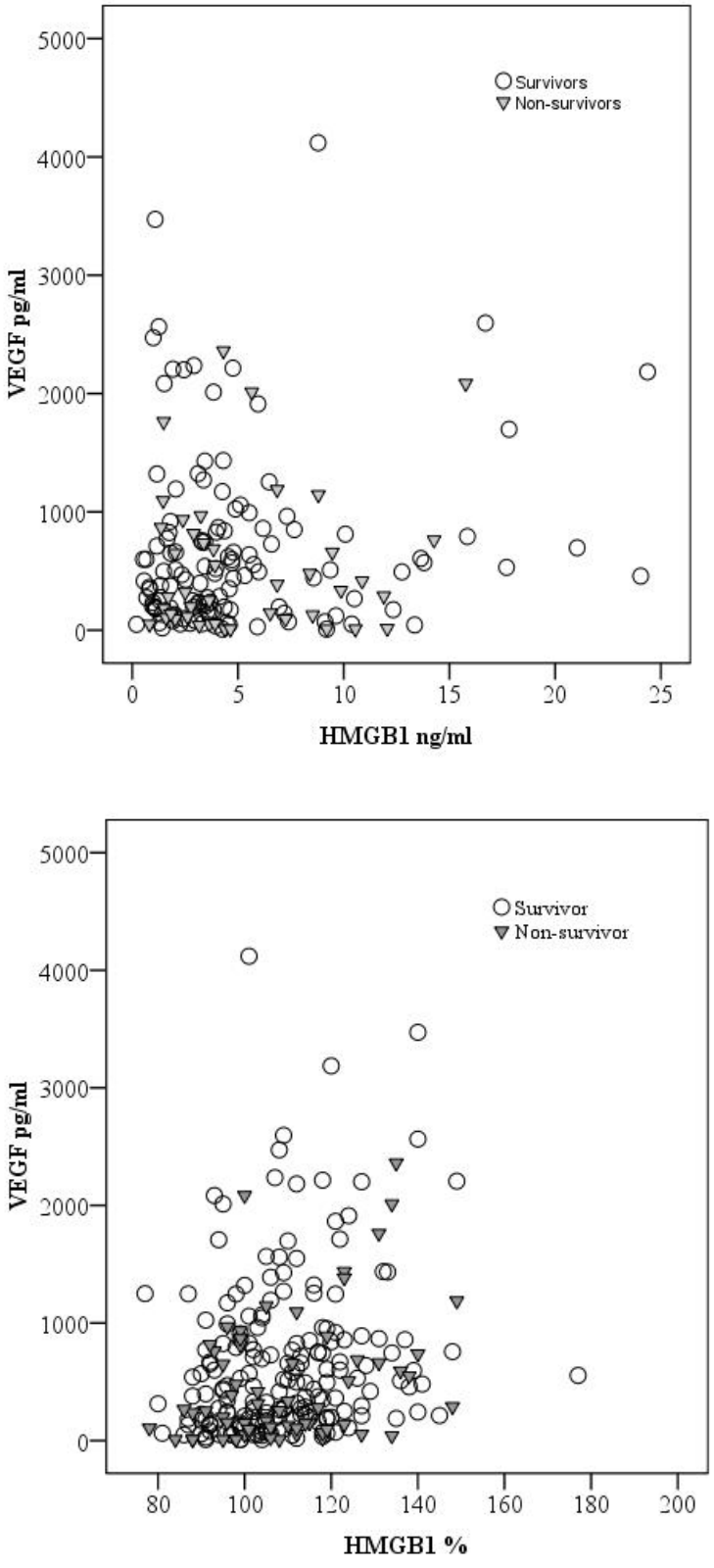



**Figure 11.** Correlation between VEGF and HMGB1 concentrations analysed by ELISA (HMGB1 ng/ml) and WB (HMGB1%) on day 0.

## 5.6 SHORT-TERM AND LONG-TERM OUTCOME (I, IV)

The ICU mortality was 15.5% and hospital mortality 28.3% in all Finnsepsis patients. Hospital mortality was affected by the number of organ failures, as in patients with one organ failure (SOFAmax score 3-4) the mortality was 11.5% but with three organ failures mortality increased to 34.0%. Age had also a strong influence on outcome, as hospital mortality was 40.0% in patients over 65 years of age and 20.4% in those under 65 years of age. Long-term mortality was also higher in age groups over 55 years than those of younger age groups (35.1% vs. 9.8%, p=<0.001). Treatment was withheld or withdrawn in 18% of patients during the treatment period in ICU. The cumulative one-year and two-year mortalities were 40.9% and 44.9% respectively. The Kaplan-Meier survival curves for one-year and two-year survival in different age groups are shown in Figures 12 and 13.

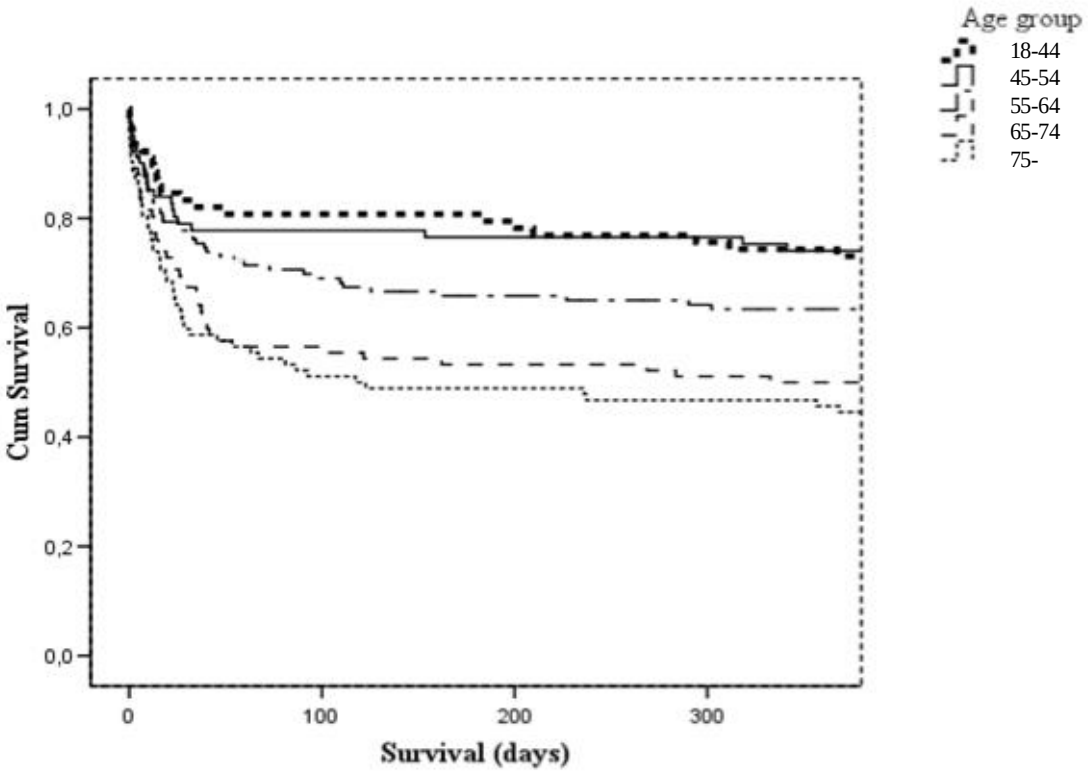



**Figure 12.** Kaplan-Meier curves for one-year survival after severe sepsis in different age groups. (Figure 3 from Study I).

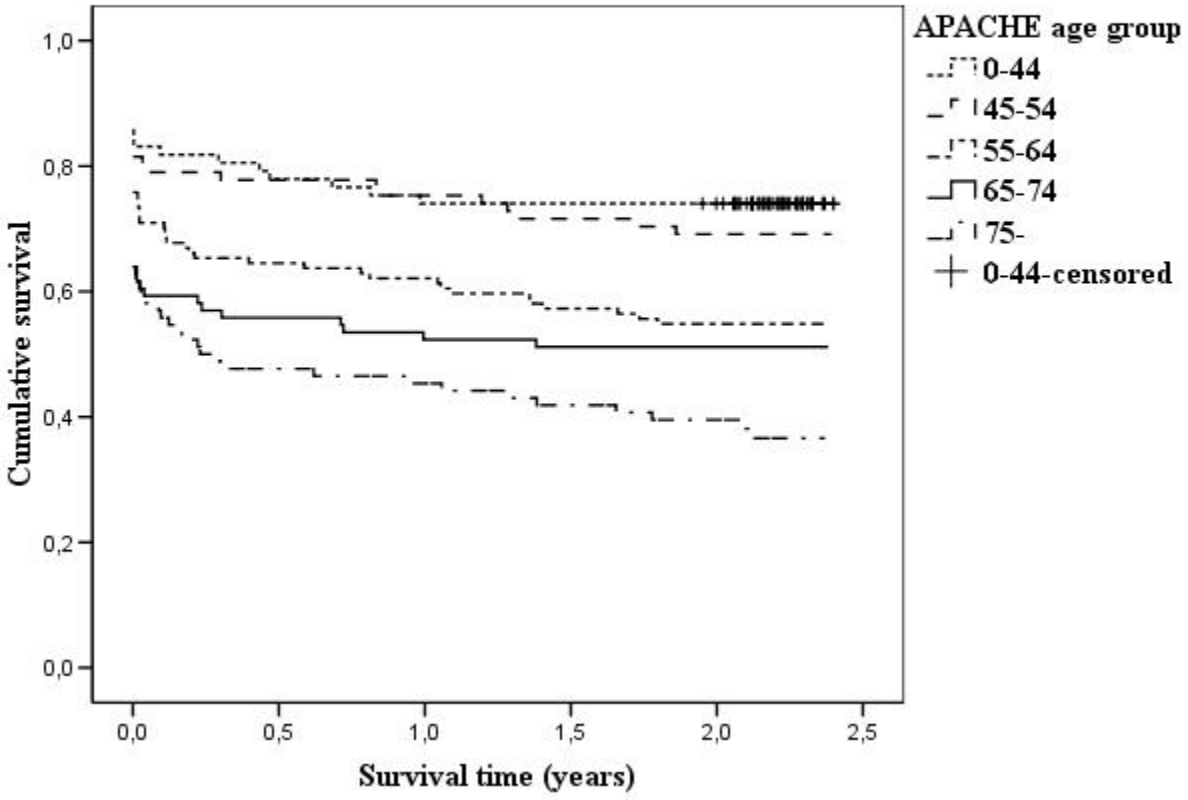



**Figure 13.** Kaplan-Meier curves for two-year survival after severe sepsis in different age groups.

## 5.7 QUALITY OF LIFE, QUALITY-ADJUSTED LIFE YEARS AND COST OF TREATMENT (IV)

*Quality of life*

Two-hundred and fifty-two patients participated in the quality of life assessment (53.6% of patients) (EQ I). The majority of the first questionnaires were completed by next of kin (61.9%). One hundred and fifty-six patients replied to the second (EQ II) questionnaire, which was sent once to 269 survivors. The preadmission EQ sum and EQ VAS were both lower than the age-and gender-adjusted references in the Finnish population. The median response time for the second QOL assessment was 17 months (IQR 16-18) after hospital discharge. The EQ sum and EQ VAS were lower in patients after severe sepsis than age- and gender-adjusted references (Figure 14). The difference between the mean EQ sum and corresponding reference value was 13 (SD 21; 95% CI, 9-16) and the difference between

the mean EQ VAS and reference value was 8 (SD 19; 95% CI, 5-11). Ninety-eight patients completed both EQ-5D questionnaires. EQ sum was also lower after severe sepsis than before critical illness in this group of patients. EQ VAS did not differ from preadmission values (Figure 15.

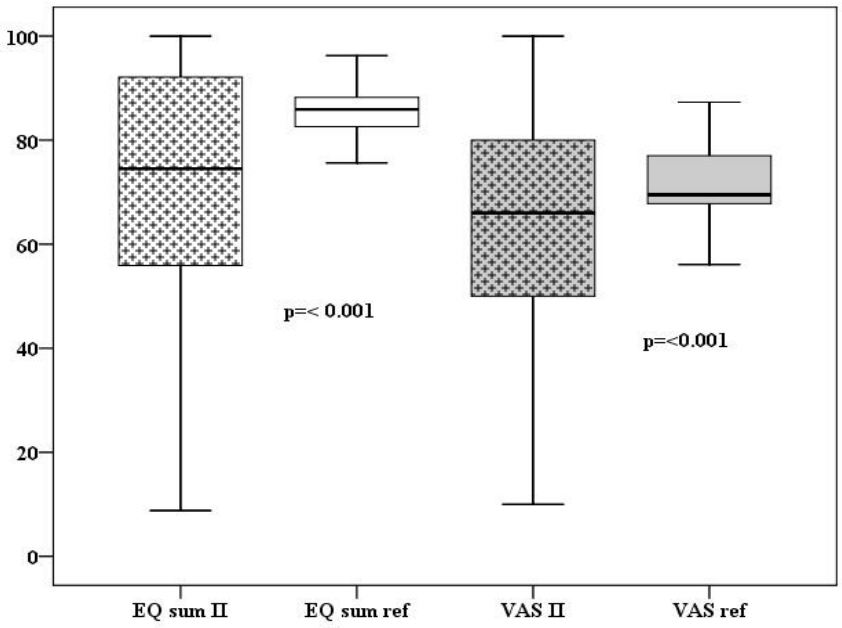



**Figure 14.** EQsum and VAS in 156 patients with EQ II responses.

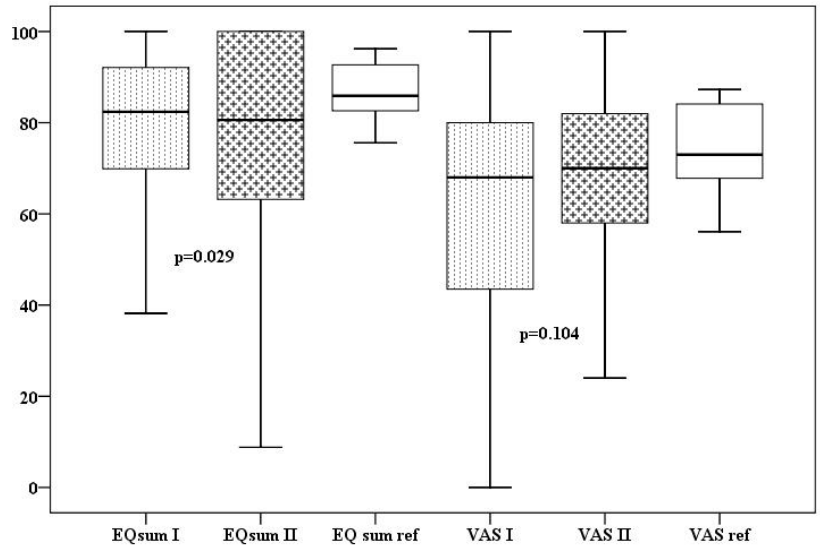



**Figure 15.** EQsum index and EQ VAS in 98 patients who completed both questionnaires (Figure 2 from Study IV).

*Quality-adjusted life years and cost of treatment*

Mean QALYs gained were 15.2 (95% CI 13.8-16.7) in surviving patients and 10.9 (95% CI 9.7-12.1) in all patients. QALYs in different age groups are presented in Figure 16. The total costs of all study patients with severe sepsis were 10,973,768 euros and the total estimated sum of acquired QALYs was 5131. The calculated cost for one QALY was 2,139 €. The costs for patients by age are presented in Figure 17. The costs varied between individual patients and very expensive treatments were found in every age group under 75 years. The mean total cost for a surviving patient with severe sepsis was 32,563 €, including mean costs of 22,915 € (70.4% of total) for intensive care and 9648 € for treatment in ordinary wards. The mean estimated cost per QALY increased in older age groups and varied from 325 € to 12,452 € (Figure 18).

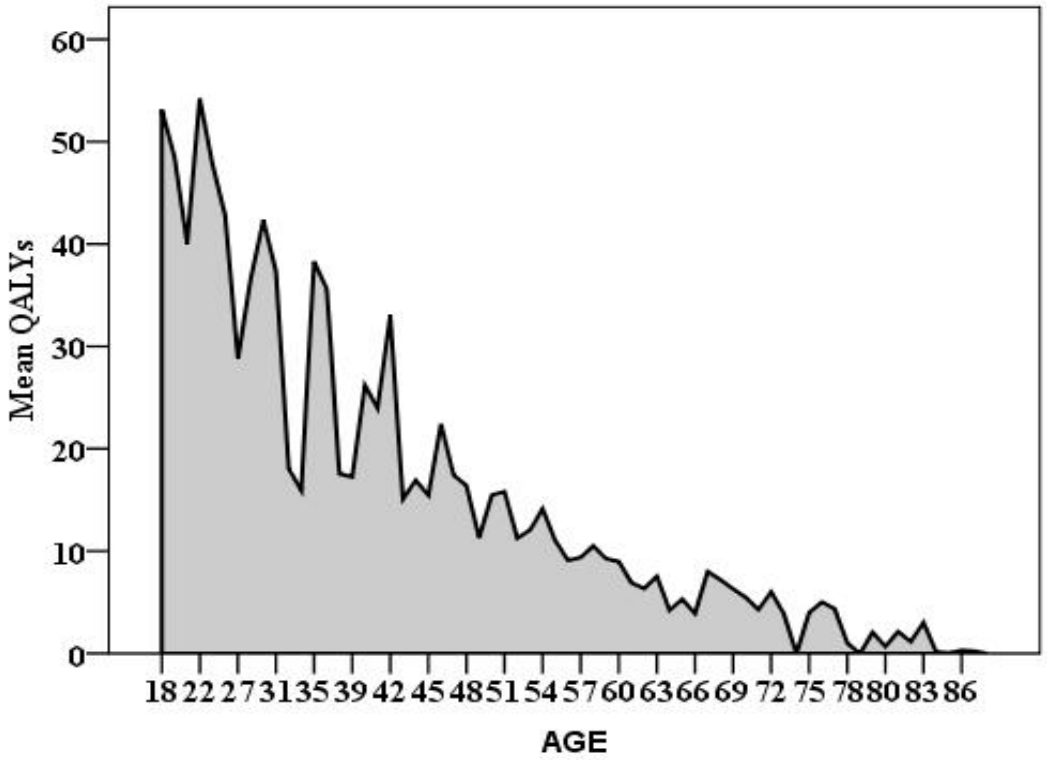



**Figure 16.** Quality-adjusted life years (QALYs) after severe sepsis by age (Figure 3 from Study IV).

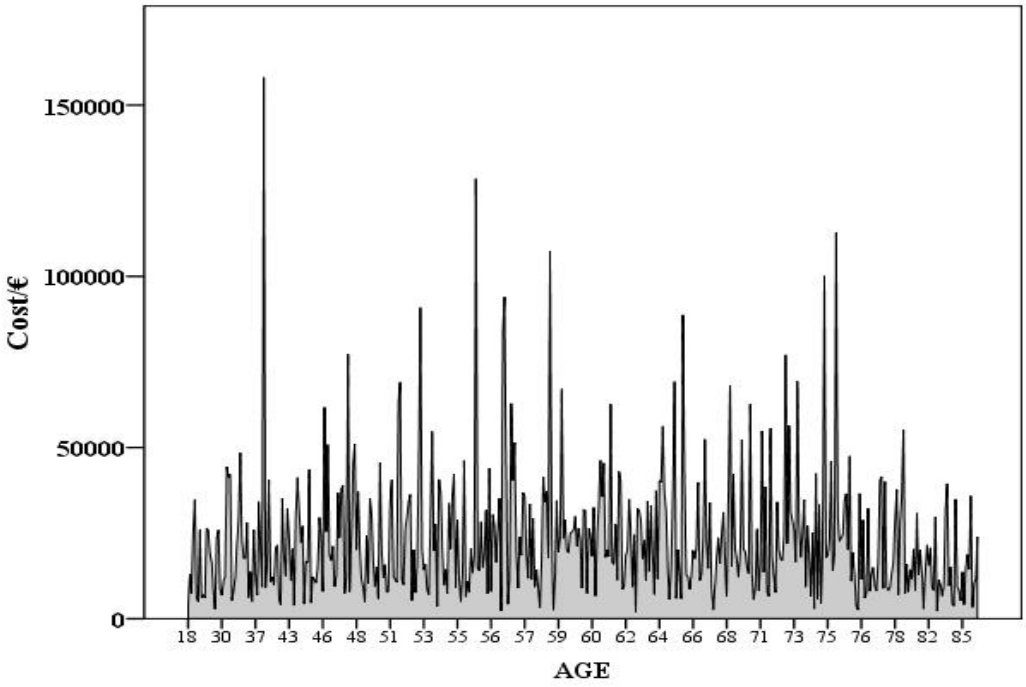



**Figure 17.** The cost of severe sepsis treatment of individual patients presented by age.

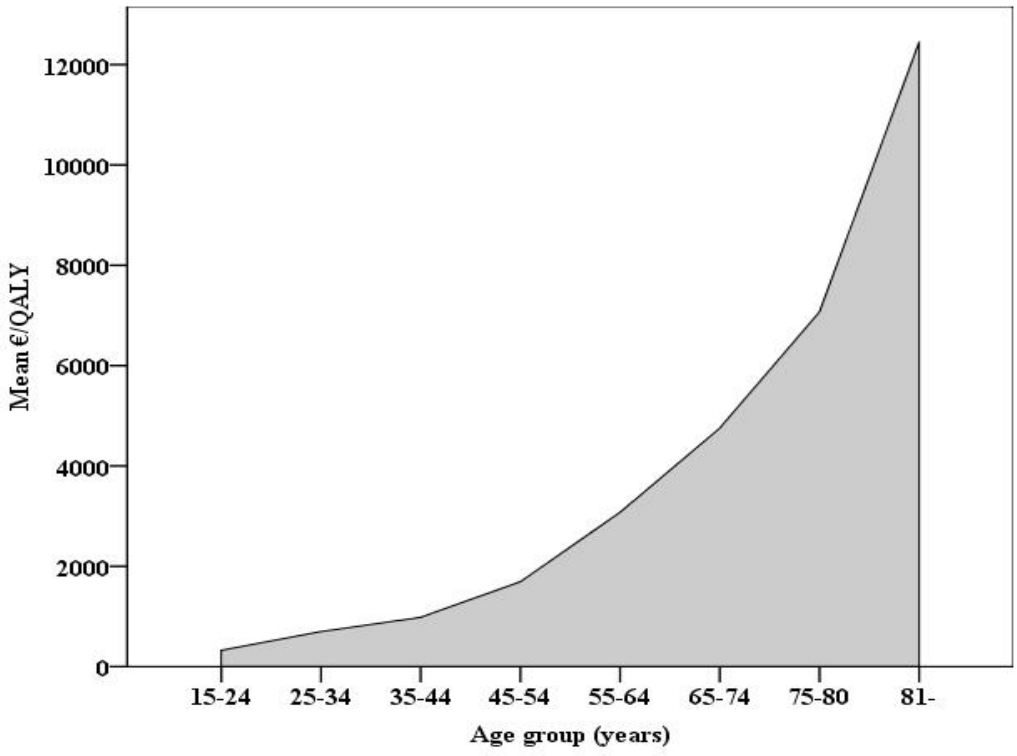



**Figure 18.** Cost-effectiveness of severe sepsis treatment (Figure 4 from Study IV).

# 6 DISCUSSION

The incidence of severe sepsis is increasing as population ages and the aim to decrease the morbidity and mortality associated with severe sepsis is a real challenge. The Finnsepsis Study was a nationwide, prospective, multicentre study with coverage of nearly 90% of Finnish adult population. Patients were identified with generally accepted and widely used severe sepsis criteria (Bone et al. 1992). Data for the Finnsepsis Study were collected locally by experienced ICU doctors and nurses and the reliability of the data was confirmed by rechecking a part of the Finnsepsis data in every sixth patient while two infectious disease specialists assessed the adequacy of administered antimicrobial treatment. We analysed two markers of inflammation in a considerable number of patients and the long-term outcome with QOL assessment was studied extensively. This study likely improved the recognition, diagnosis and treatment of severe sepsis in Finland because during the study period we specifically focused on this group of patients in Finnish ICUs. At the same time, the Finnish treatment guidelines for severe sepsis in adults were published (Finnish Anesthesiology Society 2005). Our results should be valid in an unselected adult patient population with severe sepsis. The general challenges with clinical studies are heterogeneous patient populations with various pathogens and indefinite severe sepsis onset times. The current knowledge of effective treatments in severe sepsis is still inconsistent and inadequate and under continuous re-evaluation.

## Low incidence of severe sepsis in Finland

The actual incidence of severe sepsis and associated high mortality were realized worldwide in the beginning of this millennium. The incidence found in different studies is dependent on the population, time frame and methods. The incidence of severe sepsis has varied from 0.5 to 3 cases per 1,000 inhabitants (Angus et al. 2001 a; Brun-Buisson et al. 2004). Prospective studies have been conducted usually in patients treated in intensive care and retrospective studies have also included patients treated in hospital wards. Not surprisingly, the incidence in retrospective studies (Angus et al. 2001 a; Martin et al. 2003) has been even four times higher compared to prospective studies (Finfer et al. 2004 a; Brun-Buisson et al. 2004). Before the Finnsepsis Study, the incidence of sepsis in the Nordic Countries had been studied only retrospectively in Norway and the estimated

incidence was 0.48/1,000 inhabitants (Flaatten 2004). Our results are in accordance with this retrospective incidence. The incidence was lower than previously published in countries outside Scandinavia (Padkin et al. 2003; Finfer et al. 2004 a; van Gestel et al. 2004; Engel et al. 2007), but the proportion (10.5%) of severe sepsis in all ICU admissions (472/4500) was comparable to the other countries. The low proportion of ICU-acquired severe sepsis in our study (9%) may in part reflect the difficult diagnosis of new severe sepsis during the ICU stay than the true incidence (Ylipalosaari et al. 2006).

There may be several reasons for low incidence of severe sepsis in Finland and other Nordic countries compared to the other regions in the world. First, as resistant microbes are causing increasing number of septic infections (Annane et al. 2003), Finland has long been a country with a low prevalence of methicillin resistant Staphylococcus aureus (MRSA) or other resistant microbes. Previously over 99% of Staphylococcus aureus bloodstream infections have been caused by methicillin sensitive strains (Lyytikäinen et al. 2005) but at present the number of MRSA cases continues to increase strongly (Kerttula et al. 2007). At the time of the Finnsepsis study, no multidrug-resistant microbes causing infections such as MRSA or vancomycin resistant enterococci were found. The antibiotic treatment was considered adequate in 90.2% of patients at the time the positive blood culture was taken. This effective antimicrobial treatment may also have an influence on the moderate hospital mortality rates seen in the Finnsepsis Study. Secondly, 60% of patients with community-acquired severe sepsis had previous physician contact and 20% of those patients were on antibiotic treatment at the hospital admission. Finnish health care system with health centres and general hospitals is easily accessible and it is likely that early treatment of infections affects their severity. In the recently published SOAP study, a considerable variation in the severe sepsis rates as compared to sepsis was shown between different countries. (Vincent et al. 2006). In Scandinavia, 25% of septic patients had severe sepsis compared to 45% in United Kingdom and Ireland.

Patients with severe sepsis are also treated in hospital wards. According to the Consensus criteria (Bone et al. 1992), patients with mild, short-timing organ dysfunction may fulfil the definition. In general, patients with severe sepsis should be treated in ICUs or high dependency units. A few patients fulfilling the diagnosis of severe sepsis were treated on the hospital wards of the participating hospitals during the Finnsepsis Study. Most of them were not treated in ICUs because of severe comorbidities like incurable malignancies

(unpublished data). Patients with severe sepsis are also treated in other than university or central hospitals but their number was limited to a few patients in a month according to an Internet-based query in regional hospitals without ICUs (unpublished data). Thus, it was reasonable to assess the incidence of severe sepsis only in a critical care setting. In general, prospective studies in severe sepsis are done in the ICU patient population (Finfer et al. 2004 a; Engel et al. 2007).

## Compliance to protocols and guidelines

The first Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock were published in April in 2004, 6 months before the Finnsepsis Study started (Dellinger et al. 2004). Many recommendations were based on studies published a few years earlier (Bernard et al. 2001; van den Berghe et al. 2001; Rivers et al. 2001; Annane et al. 2002). The adoption of some of these recommended treatments was incomplete during the study. This is not surprising, since there are many studies concerning the difficulty of implementing clinical guidelines into practice. Many of these reports assess the use of low tidal volumes (Young et al. 2004; Rubenfeld et al. 2004) but the problem applies to any recommendation or guideline (Cabana et al. 1999). However, after the Finnsepsis Study, many of these above mentioned studies concerning treatment in intensive care in general or specifically in patients with severe sepsis have been questioned and re-evaluated. New studies failed to show benefit (Sprung et al. 2008) or revealed harmful effects (Brunkhorst et al. 2008). This discrepancy has indeed confused the clinician at the bedside.

At the time of our study, low dose corticosteroid treatment was recommended for septic shock in general but it was administered to less than half (41.3%) of the eligible patients. ACTH stimulation test was done on only a few patients (6.6%). Nowadays, stimulation test is not recommended to identify those patients who should receive corticosteroids (Marik et al. 2008). The compliance with the corticosteroid treatment might be considered only moderate according to the guidelines obtained at that time.

In our study, the mortality did not differ in septic shock between patients with or without low dose corticosteroid treatment. This concurs with the results in the Corticus Study

showing no difference in mortality between patients with septic shock receiving corticosteroids or placebo (Sprung et al. 2008). The patients in the Corticus Study were less sick than in the first study to show benefit in septic shock (Annane et al. 2002). At present, patients with vasopressor-resistant septic shock may be treated with low dose corticosteroids (Marik et al. 2008). In our study, the number of patients with vasopressor-resistant septic shock and low dose corticosteroid treatment were not definitely registered.

Treatment with activated protein C was given to one third of the patients with probable indication according to Finnish recommendations published in 2003, which include DIC and at least one other acute organ dysfunction (Pettilä et al. 2003). According to general concept of the use of activated protein C in patients with at least two organ dysfunctions and APACHE II score 25 or higher, activated protein C was given in 7% of these patients in the Finnsepsis Study. This frequency can be considered rather low. However, contraindications of the treatment such as recent surgery were not assessed. After our study, the negative results using rhAPC in less severely ill patients were published (Abraham et al. 2005) and a Cochrane review showed no evidence to advocate rhAPC in patients with severe sepsis (Martí-Carvajal et al. 2008). New placebo-controlled trials with rhAPC like PROWESS-SHOCK (Finfer et al. 2008) and APROCCHS http://www.clinicaltrials. gov/ct2/ show/NCT00625209) will hopefully settle the controversy surrounding this drug. Meanwhile, according to the Finnish guidelines rhAPC may be administered to those patients with severe sepsis complicated by developing MODS including DIC.

Tight glucose control was quickly and widely accepted also to be applied in patients with severe sepsis after publication of the study results (van den Berghe et al. 2001). This was the first study to show any mortality reduction after failures in specific sepsis treatment trials (Abraham et al. 1998; Dhainaut et al. 1998). Treatment with insulin was considered cheap and it was easily adoptable, because nurses at the bedside carried out the treatment. In our study, blood glucose level was set at under 6.5 mmol/l in 41.5% and under 8 mmol/l in 53.6% of patients. However, we did not analyse the glucose levels during the treatment. The risk of hypoglycaemia with strict glucose control became soon evident. A recent meta-analysis found no reduction in mortality in patients with critical illness but showed again the increased risk of hypoglycaemia (Wiener et al. 2008). The results of the NICE-SUGAR

study will be shown in 2009. We need to establish the best risk-to-benefit ratio in glucose control.

At present, only few separate treatments have been found to affect outcome. The early and proper antibiotic treatment is one example of a simple intervention that has been shown to reduce mortality (Leibovici et al. 1998; Kumar et al. 2006; Varpula et al. 2007). Instead of individual treatments, bundles consist of various goal-directed interventions and procedures to be performed simultaneously or in certain time limits depending on the disease process. The implementation and good compliance with treatment bundles and protocols can decrease mortality in severe sepsis (Gao et al. 2005; Kortgen et al. 2006; Ferrer et al. 2008).

Protocols even from successful trials showing mortality reduction are adopted slowly, if ever (Kalhan et al. 2006; Umoh et al. 2008). It may be difficult to comply fully with strict protocols and new knowledge has to be adapted at the bedside for individual patients. In addition, protocols in randomised, prospective studies can lead to patients receiving a completely different level of treatment than they would get without the study protocol. For example, patients with ARDS might have been randomised to a group receiving higher tidal ventilation than without the trial. This can make interpretation of the study difficult and may even threaten the study safety (Deans et al. 2007).

## The role of HMGB1 and VEGF in severe sepsis

In our patients, high HMGB1 levels were not associated with different types of organ dysfunctions and unexpectedly, patients with the most severe organ failure had lower HMGB1 concentrations than patients with milder organ dysfunctions. In addition, non-survivors had lower serum VEGF concentrations than survivors and severe renal and haematological failures were also associated with low VEGF. Neither HMGB1 levels nor VEGF concentrations predicted mortality.

Our HMGB1 and VEGF measurements were studied in altogether 250 patients. Many studies with divergent results have been conducted in studies with distinct difference in size. First VEGF measurements in septic patients were done in 18 (van der Flier et al.

2005) and first HMGB1 measurements in 25 patients (Wang et al. 1999). Our patients with HMGB1 and VEGF measurements are a representative group of patients with severe sepsis.

The timing of laboratory samples to detect mediators with various half-lives is challenging. Once elevated, no significant increase or decrease in HMGB1 levels has been found within one week after onset of severe sepsis (Angus et al. 2007). This confirms that the two samples taken from our patients within 72 hours are representative in measuring HMGB1. A different time pattern may partly explain differences between the two small studies and our study. The peak VEGF concentrations occurred in the first 24 hours and VEGF levels remained elevated for up to several days (Yano et al. 2006. van der Flier et al. 2005). In our study, more than half of our patients (56.7%) had further increasing VEGF concentrations over the first 72 hours. As pointed out earlier, the severity of illness and the exact onset time of severe sepsis vary in clinical studies and may have had an influence on the VEGF levels. No adequate data exists to define which time interval should be used for the analysis of VEGF and for this study, 3 days was used.

Experimental studies can determine the exact time for inducing sepsis e.g. with LPS injection. In real life, the only exact time point we are aware of is the hospital admission time. Conflicting results in different studies may reflect the fact that patients have been in different phases of their sepsis or they present with different types of sepsis. Patients with community acquired severe sepsis (Angus et al. 2007) probably differ from patients with severe hospital acquired infections. Our patient population was mixed, with 58% of patients having community-acquired infections.

We had some difficulties in measuring HMGB1 levels with WB which is a time consuming and semi-quantitative method. We failed to obtain pure HMGB1 for a control and we used somewhat arbitrary units to report the results. The WB method has limitations because of possible cross-reaction with light chains of immunoglobulins (Sunden-Cullberg et al. 2005) and indeed, in some of our healthy controls elevated HMGB1 levels were measured. When the ELISA method for HMGB1 measurement became available, we decided to re-analyse the samples of a subgroup of our patients and healthy controls. The HMGB1 levels in healthy controls were very low, but could still be detected. HMGB1 levels measured with WB and HMGB1 concentrations measured with ELISA showed no

correlation in an individual patient analysed by Spearman's correlation. However, the primary results obtained by Western blotting were confirmed by ELISA concerning the mortality and organ dysfunction. Some HMGB1 studies have been done with WB (Wang et al. 1999; Angus et al. 2007) and others with ELISA (Gaïni et al. 2007; van Zoelen et al. 2007). These differences in methodology may be confounding and must be taken into account when drawing comparisons between studies with different methodologies.

## Short and long-term outcome

The ICU mortality in our study may be considered low compared to other studies reporting mortalities of over 20% (Martin et al. 2003; Finfer et al. 2004 a) and the cumulative hospital mortality of 28.3% is similar or lower compared to other surveys (Martin et al. 2003; Flaatten 2004). Age has been shown to be an independent risk factor for sepsis mortality (Martin et al. 2006) and the effect of age was shown also in our study: the patients over 65 years had twofold mortality compared to younger patients (40.5% vs. 20.4%). The number of failing organs affected strongly the mortality. The hospital mortality in patients with at least two organ failures has been four-hold compared to patients with only one organ failure (Guidet et al. 2005). The hospital mortality varied between 38% and 71% in patients with at least two severe organ failures in our study. However, the in-hospital increase in mortality over 10% is remarkable, even if the approximate hospital mortality in severe sepsis has been about 30% (Angus et al. 2001 a; Sundarajan et al. 2005). Restrictions of care such as withhold or withdraw of the treatment was done in 18% of the patients. Severe co-morbidities or poor recovery from the underlying disease have impact on hospital mortality. Nearly in half of those patients the withhold decision was done at the discharge from the ICU thus refusing the possible re-admission. The cumulative hospital mortality in our study was in concordance to the fact that limitations of care in the ICU are the most powerful predictor of post-ICU mortality (Azoulay et al. 2003). On the other hand, the hospital mortality was low (14.5%) for those patients, whose treatment was not restricted in ICU or at discharge.

Most patients who die in the first year after severe sepsis seems to die in the first two months after hospital discharge. The cumulative one-year mortality in the Finnsepsis Study was 40.9% and 2-year mortality was 1.5 times higher than the hospital mortality. Even if

the survival was poor, the mortality was lower than previously published one-year mortality of 51.4% (Weycker et al. 2003) and two-year mortality of 67% (Korošec Jagodič et al. 2006). In general, no excess mortality has been found after 2 years in patients recovering from critical illness (Niskanen et al. 1996; Flaatten and Kvåle 2001). Former sepsis trials have looked only the short term mortality as an endpoint (Bernard et al. 2001) but forecoming trials should look at least 3-month or 6-month survival as their outcome. To increase long term survival after severe sepsis, we should pay more attention to the patient selection, post ICU care and rehabilititation.

## Quality of life

The long-term survival with acceptable QOL should be one of the important outcomes after critical illness (Angus et al. 2003 a). In our study, the surviving patients had lower QOL than the age- and gender-adjusted population even 1.5 years after intensive care discharge. However, the estimation of mean gained QALYs for a surviving patient was 15.2 years, which can be considered a reasonable outcome after a life threatening disease.

QOL was already lower in most of our patients before the episode of severe sepsis compared to the general population. Our gender- and age-adjusted reference values in Finnish population for QOL assessment were measured over 10 years ago (Ohinmaa and Sintonen 1996). Newer values are not available but this has hardly influenced our results. QOL before critical illness has been evaluated in some studies and poor QOL has been correlated with worse outcome (Cuthbertson et al. 2005. Hofhuis et al. 2007). No previous studies exist concerning pre-illness QOL in patients with severe sepsis. Our results are in accordance with other pre-illness QOL assessments, because before critical illness non-survivors had lower EQ-5D index and VAS scores than survivors.

Quality of life has been studied increasingly among critical care in recent years, but most studies have investigated critically ill patients in general. The number of septic patients has been small or cannot be identified from the study population (Flaatten and Kvåle. 2001; Graf et al. 2005; Kaarlola et al. 2006). In general, our results for QOL after severe sepsis are in agreement with those of other studies. QOL assessments show poorer QOL in patients after severe sepsis compared to either controls like patients with trauma or to an

age- and gender-adjusted general population (Heyland et al. 2000; Granja et al. 2004; Korošec Jagodič et al. 2006). Severe sepsis causes organ dysfunctions with a possible slow recovery in surviving patients. Patients with acute respiratory distress syndrome have impaired QOL one year after critical illness (Angus et al. 2001 b) and acute renal failure has a strong impact on QOL and worsening the long term outcome (Åhlström et al. 2005). Major trauma and severe sepsis both cause reduction of QOL, but no difference was found between the groups after two years (Korošec Jagodič et al. 2006).

Our QOL assessment was conducted at median 17 months after hospital discharge, which we considered an adequate time period to recover from severe sepsis and its sequelae. In other studies the time interval for QAL assessment has often been between 6 and 12 months (Niskanen et al. 1999; Granja et al. 2004; Hofhuis et al. 2007). However, the time for QOL assessment in the general ICU population has varied from the time of hospital discharge (Hofhuis et al. 2007) to as long as 6-12 years after ICU treatment (Flaatten and Kvåle 2001; Kaarlola et al. 2003).

Earlier clinical studies did not evaluate QOL, but the AT III study was one of the first clinical sepsis trials to publish quality of life results in study patients (Rublee et al. 2002). At present, QOL assessment is recommended to be evaluated in all clinical trials (Angus et al. 2003 a).

Assessment of QOL is more reliable using validated QOL instruments in large unselected cohorts with extensive and reasonably long follow-up and with comparison to pre-ICU baseline evaluation (Dowdy et al. 2005). We used in our patients the generic EQ-5D measurement (The EuroQol Group 1990), which is recommended to be used in patients with critical illness (Angus et al. 2003 a). EQ-5D is also suitable for cost utility analyses. Our follow-up time was long enough, but the response rate in the second QOL assessment (156 patients, 58% of the surviving patients) could have been higher. The response rate often is reasonable low in this type of studies. Approximately 50-60% of surviving patients have responded QOL assessment done by a telephone interview (Eddleston et al. 2000; Granja et al. 2004). As we posted the questionnaire, the response rate may be considered satisfactory.

The mean cost for one QALY estimated in the Finnsepsis study definitely meets cost-effectiveness criteria. Recently published estimates of costs per one QALY after severe sepsis have compared activated protein C to placebo and standard care and costs per QALY vary from $7,800 to -$958, 000 (Manns et al. 2002; Fowler et al. 2003; Angus et al. 2003 b). In our patients, the costs per QALY increased by a factor of 40 with increasing age from the youngest to the oldest patients. However, QALYs in the elderly patients are not equal compared to younger patients, because even with good quality of life QALYs will be low due to the shorter life expectancy. The mean costs per QALY were within reasonable limits for all patients and our mean calculated costs per survivor and per QALY seem to fall within the lower range of previously published data (Ridley et al. 2007; Talmor et al. 2008).

The estimation of QALY is extremely sensitive to changes in distribution of age in treated population, mortality rate and gained QOL. In our calculations, we used zero QALYs for hospital non-survivors. The long-term QOL was measured by the validated measurement and the mean age- and gender-matched values of respondents were used for the estimation of QOL also for non-respondents. Even if these assumptions were made, we feel that our calculation provides a reliable estimation. In addition, this method has been used in an earlier study (Kaarlola et al. 2006). We also assumed for the QALY assessment that the QOL of the study patients would be unchanged after 17 months, and that there would be no excess mortality after 2 years, based on the previous Finnish study (Niskanen et al. 1996).

The costs of critical care were calculated by using the real costs in Finnish ICUs. The ICU cost estimation is based on TISS point cost based on all patients treated in Finnish ICUs, not only on patients with severe sepsis and thus we feel this method is reliable and reproducible. The cost for a treatment period in hospital ward was more difficult to estimate, because hospitals have only average prices for ward treatment or the price is based on a diagnosis-related group. As the majority of hospital costs (in average 70%) are from intensive care, the relative impact of ward costs to final calculations was supposed to be quite small. Social security, various insurance costs or rehabilitation costs were not evaluated in this study.

## Limitations of the study

Our study has some limitations. Not all Finnish ICUs participated in our study, but because 90% of Finland's university and central hospitals participated, we consider it highly representative. During a limited 4- month study period it is possible that seasonal changes in the occurrence of diseases influence the observed incidence. However, no differences in distribution of patients to different diagnostic categories have been found between the seasons in Finland (Reinikainen et al. 2006). A small minority of patients with severe sepsis may have been treated in small regional hospitals and on wards, which were not included in the study. In addition, two ICUs treating transplant patients with immunosuppression and susceptible to severe sepsis did not participate in the study. It must be noted that the availability of beds in ICU wards may affect to both the severity of disease during inclusion and the quality of care. In our study, we found only less than 1% patients with severe sepsis who were treated outside ICU because of shortage of ICU beds.

The blood samples for HMGB1 and VEGF were taken only at two time points, and patients could have been at different phases in the course of their sepsis. However, onset of sepsis can only be estimated and the only definite time point is the time of the hospital admission. The first samples were taken, when patients met the criteria for severe sepsis or septic shock and the second sample was taken 72 hours thereafter. We believe that 72 h time window was sufficient to detect a HMGB1 or VEGF response if present. HMGB1 levels increase after 8 h in experimental endotoxin shock (Andersson et al. 2000) and stay elevated at least one week (Sunden-Cullberg et al. 2005). The half life of VEGF is short (Eppler et al. 2002), but studies have shown increased VEGF levels up to 29 days, indicating sustained VEGF production in patients with severe sepsis (Yano et al. 2006).

The quality of life assessment in the first query comprised 53.6% of the Finnsepsis study patients, and the second query was answered by only 56.1% of surviving patients. Two large hospitals failed to require consents for QOL assessment, which was related to the only moderate response rate. However, the demographic data did not differ between respondents and non-respondents. Patients without response for the first questionnaire had a higher ICU and hospital mortality. Therefore, the preadmission QOL could have been overestimated. Those not responding to the second questionnaire were younger patients with shorter ICU stays compared to patients with second responses. This may affect QOL,

leading to an underestimation of QOL following the septic episode. The sample size for coupled answers was quite small compared to all surviving patients (35%). Queries were answered in part by patients' next of kin but relatives can reliably complete QOL questionnaires on behalf of the patient (Hofhuis et al. 2003).

## Clinical implications and future perspectives

Timing seems to be most important factor in the treatment and outcome in severe sepsis. The onset of infection and body's inflammatory response should be identified as early as possible, but signs of severe sepsis may not be evident at hospital admission. At present, we do not have biomarkers, which could early and reliably detect patients with progressive severe sepsis or with increased risk of death. The early and goal-directed treatments have changed our focus from intensive care to emergency room (Rivers et al. 2001) and on wards (Ferrer et al. 2008). The right timing of adequate antimicrobial treatment is essential for survival especially in septic shock (Kumar et al. 2006). The prompt administration of broad-spectrum antimicrobial treatment has the equal cost as the delayed administration, but it can increase survival and is certainly cost effective.

The long term survival with the quality of life assessment has become most important outcome after critical care. Ageing population increases the need for intensive care in the future and we have to focus on treating those patients with acceptable long term outcome. Quality of life assessment after intensive care with EuroQol-5D has been implemented in Finnish Quality Consortium data in 2008.

Despite active investigation, evidence-based treatments and protocols are infrequent in intensive care. Studies in intensive care in general have had conflicting results for example in renal replacement therapies (Ronco et al. 2000. VA/NIH Acute Renal Failure Trial Network 2008). It takes several years to complete a large, randomized, prospective study. Groups of patients may differ from previous studies (Annane et al. 2002. Sprung et al. 2008) or other treatments may have changed during study years. Heterogeneous patient population complicates the interpretation of study results in a specific patient group such as severe sepsis. Future multi-centre studies should focus on specific patient groups to get best applicable knowledge for clinical practice.

The Finnish nationwide performance on the treatment of severe sepsis will be re-investigated in the future Finnsepsis II study with the reproducible methodology of the first study. This study may include the testing of new biomarkers or markers of gene polymorphism giving a new insight into the recognition of patients with increased risk for severe sepsis. In addition, a rapid pathogen regocnition for right-timed and adequate treatment in increasing survival should be tested.

# 7 CONCLUSIONS

Based on these studies the following conclusions can be drawn:

1. The incidence of ICU-treated severe sepsis in Finland is 0.38 per 1,000 of adult population, which is lower than previously published in USA, Europe or Australia. Respiratory failure requiring ventilatory support, cardiovascular failure and acute renal failure were the most common severe organ dysfunctions associated with severe sepsis. Prevailing recommended therapies were used for suitable patients in only 15%-41% of cases. Cumulative ICU, hospital, and 1-year mortalities were 15.5%, 28.3% and 40.9%, respectively. The outcome of severe sepsis in Finland is comparable to that reported for other countries.

2. Serum HMGB1 concentrations are moderately elevated in patients with severe sepsis, but they do not differ between survivors and non-survivors and do not predict organ dysfunction or hospital mortality.

3. Serum VEGF concentrations are elevated in patients with severe sepsis compared to healthy controls. However, low concentrations are associated with severe haematological and renal dysfunctions. Although non-survivors have significantly lower levels than patients with better outcome, VEGF concentrations do not predict hospital mortality.

4. Cumulative two-year mortality after severe sepsis is high (44.9%) and QOL is poorer after severe sepsis than in reference population as assessed by EQ-5D. However, the estimated mean QALY (15.2 years) for the surviving patient is reasonable and as the calculated cost for one QALY is only 2,139€, intensive care in patients with severe sepsis is considered cost-effective.

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# ORIGINAL PUBLICATIONS

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